PRELIMINARY REPORT ON THE EARLY HISTORY OF THE EGG AND EMBRYO OF CERTAIN HYDROIDS.

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Within the last few years some of the hydroids (*Pennaria, Clava leptostyla, Eudendrium, Tubularia crocea*) have been described as differing widely in their processes of maturation, fertilization, and early cleavage from what we have come to think of as typical. Other members of the same group (*Tubularia mesembryanthemum, Clava squamata, Hydra, Gonothyrea, Æquorea, Tiara, Gonionemus*) have been described as perfectly typical. That certain of the hydroids should conform to the type and others not, seemed improbable. The fact of the low organization of the group which is used by Hargitt to account for such variation seemed hardly sufficient. On this account at the suggestion of Professors Morgan and Wilson, I undertook the reëxamination of two of the forms, *Pennaria* and *Clava leptostyla*, which varied most from the type, to discover if possible the relation between the aberrations described in these forms and the usual type. The results on *Pennaria* were worked out in the winter of 1907 at the Zoölogical Laboratory of Columbia University, on material collected at Woods Hole during the previous summer. Those on *Clava* were obtained in the summer and autumn of 1908.

A brief review of Hargitt's work on *Pennaria* and *Clava leptostyla* will be necessary to recall the points which I wished to clear up. In both these forms, according to his results, the processes of maturation and fertilization of the egg are very obscure and incapable of demonstration. The germinal vesicle just before the time that maturation should take place moves to the periphery of the egg where it loses its staining capacity, the nuclear membrane breaks down, and the nuclear substance becomes diffused throughout the egg, where it is no longer recognizable, due probably to some chemical or physical change in the egg. The

maturation and fertilization processes were supposed to take place while the chromatin is in this unstaining condition, the sperm having also lost its staining capacity after entering the egg. The first evidence of nuclei in the egg after the disappearance of the germinal vesicle was found in the appearance of " nuclear nests " or groups of small vesicles scattered throughout the egg, several such nests usually occurring in an unsegmented egg. In *Pennaria* not less than four such nests appear simultaneously indicating four centers of nuclear reconstruction. In *Clava*, as I understand the description, this condition of several nuclear groups occurs occasionally in an unsegmented egg. More often, however, eggs were found already segmented with a single resting nucleus in each cell which I take it were believed to arise as described for *Pennaria*. This reappearance Hargitt thinks is explicable on the ground that after fertilization the chemical or physical conditions again change so that the chromatin once more responds to stains. The chromatic material that was scattered at the time the nucleus disappeared collects again, forms vesicles which especially in *Pennaria* occur in groups or nests, each nest finally fusing into a single nucleus. Thus a syncytium arises without mitosis and with no apparent evidence of maturation or fertilization having taken place in the egg. In *Pennaria* after these nuclear groups are formed, nuclear proliferation is by mitosis. In *Clava leptostyla*, however, up to the sixteen-cell stage Hargitt describes nuclear proliferation by amitosis, later cleavages being mitotic.

The points in question accordingly are: (1) The nature of maturation and fertilization processes, (2) the formation of nuclei *de novo*, and (3) the *rôle* of amitosis and mitosis in early cleavages.

Pennaria and *Clava leptostyla* were preserved at Woods Hole where Hargitt obtained his material. Also the same killing fluids and stains were used so that the question of method is eliminated. Material was killed every hour of the day and night and every half hour in the early morning hours which proved to be the most important period.

I found in both *Pennaria* and *Clava leptostyla* that in material killed between the hours of 4 and 6 A. M., it was possible to

demonstrate the maturation processes and that they take place with the utmost exactness and in the typical manner, as reference to the figures will show. Fig. 1 shows the germinal vesicle of an egg of *Pennaria* in which the inner wall is breaking down, the nucleolus passing into the cytoplasm where it is lost, while the chromatin is grouping itself around the periphery of the nucleus to form the chromosomes. Figs. 2 and 11 are nearly corresponding stages of the first polar spindle in *Pennaria* and *Clava leptostyla* respectively. In both forms the chromosomes are evidently bipartite and the number is determined to be one half the somatic number. In the figure of *Pennaria* (which is in the late prophase) the spindle has not yet swung around into position. A comparison of Figs. 3 and 12 shows again practically identical conditions of the second polar spindle in the two forms. The first polar body lies outside the egg, the second polar spindle is in the late anaphase. I have found numerous intermediate stages. Figs. 4 and 13 show corresponding stages of the reconstructed egg nucleus with the polar bodies lying outside the egg.

Figs. 5 and 6 give two stages in the fertilization of *Pennaria.* The two-germ nuclei lying side by side at the periphery of the egg later move toward the center of the egg where they form the fusion nucleus at the ends of which astral radiations appear. The origin of the first cleavage spindle is not determined. For lack of proper stages the fusion of the two-germ nuclei has not been demonstrated with certainty in *Clava leptostyla.*

From this point forward the two forms differ slightly. In *Pennaria,* after the first cleavage the two nuclei are reconstructed by the formation of chromosomal vesicles as shown in Figs. 7 and 8. For some reason, possibly the rapidity of nuclear divisions, the chromosomal vesicles often fail to fuse into a single nucleus but give rise to a "nuclear nest" which subsequently gives rise directly to the chromosomes of the following cleavage figure. Fig. 9 shows two such vesicles passing on to a spindle, one vesicle already broken up into the individual chromosomes, the other still in the vesicular stage. Fig. 10 shows an equatorial view of such a group of vesicles, some of the chromosomes already forming an equatorial plate.

A shortening of the resting stage between the nuclear divisions,

it seems to me, might account for Hargitt's interpretation of the "nuclear nests." The rapidity of nuclear division is accompanied by slow cytoplasmic division so that the former constantly outruns the latter, the result being that an unsegmented egg often contains several such groups of vesicles or "nuclear nests."

In *Clava* the cytoplasmic cleavage does not lag so far behind the nuclear division, but in fact keeps pace with it. For this reason it was possible as shown by Figs. 14, 15, 16, 17, 18 and 19 to demonstrate the first cleavage spindle, and the successive passage of the two-cell stage into the four-cell, eight-cell and sixteen-cell stages. The nuclear reconstruction takes place here again by the formation of chromosomal vesicles; but *Clava* differs from *Pennaria* in that, as a rule, the vesicles all fuse into a single nucleus between successive cleavages So far as I am able to tell as yet, it seems probable that the cleavage in *Clava* is fairly regular.

Summary.

In the two hydroids (*Pennaria* and *Clava leptostyla*) under question, the maturation and fertilization processes take place in a perfectly typical fashion and form no exception to the general rule in this regard.

The conclusion that the "nuclear nests" indicate the formation of nuclei *de novo* is shown to be untenable. The occurrence of these nests is explained by the conditions of nuclear reconstruction after cleavage, the chromosomal vesicles failing to fuse between successive divisions in *Pennaria* and the cytoplasmic division lagging behind nuclear division gives a syncytium with several nuclear groups.

Maturation and the early cleavages take place by means of mitosis and not amitosis. No evidence whatever of amitotic division was found.

My results regarding the maturation and fertilization phenomena make it very probable that Hargitt's failure to observe these stages was due simply to the fact that the eggs were not obtained at the right time of day. In eggs collected at the proper time (4–6 A. M.) there is no difficulty in proving the typical stages of maturation and fertilization.

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Explanation of Plate I.

Pennaria. All drawings × 1,300.

Fig. 1. Early prophase of germinal vesicle of egg of *Pennaria;* the inner nuclear wall is breaking down; the nucleolus passing into the cytoplasm; the chromosomes forming.

Fig. 2. Later prophase of first polar spindle of egg of *Pennaria;* some of the chromosomes bipartite; some quadripartite. Reconstructed from two consecutive sections.

Fig. 3. Anaphase of second polar spindle of egg of *Pennaria.*

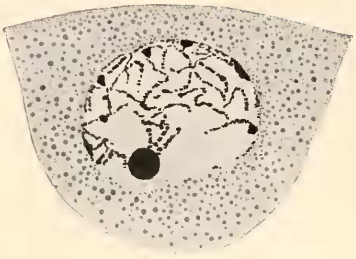
Fig. 4. Female germ nucleus and polar bodies in egg of *Pennaria;* chromatin is in fine reticulum.

Fig. 5. Fertilization of *Pennaria* egg; male and female germ nuclei at the periphery of the egg and of equal size. Reconstructed from three consecutive sections.

Fig. 6. Egg of *Pennaria* showing the fusion nucleus with astral radiations at either end of the nucleus.

Fig. 7. Telophase of 1st cleavage of egg of *Pennaria;* nuclear reconstruction by the formation of chromosomal vesicles.

Fig. 8. "Nuclear nests" formed by the partial fusion of the chromosomal vesicles lying at the ends of the spindle (probably second or third cleavage). *Pennaria.*



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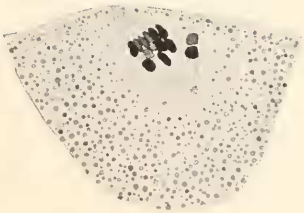

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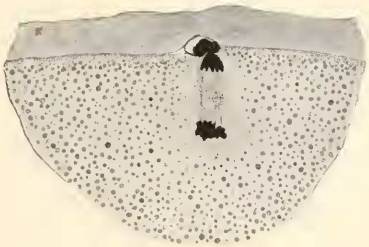

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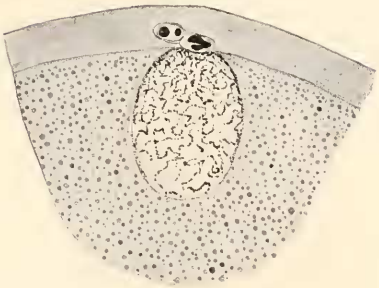

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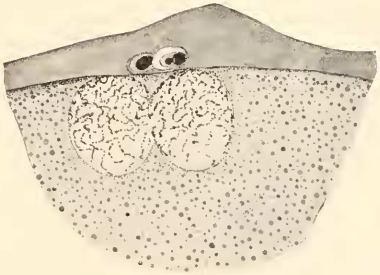

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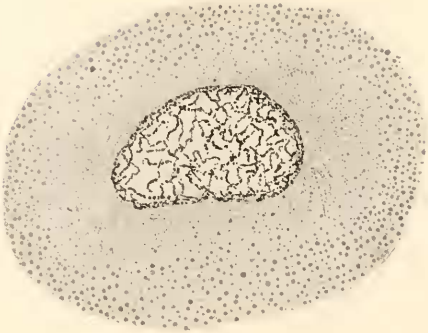

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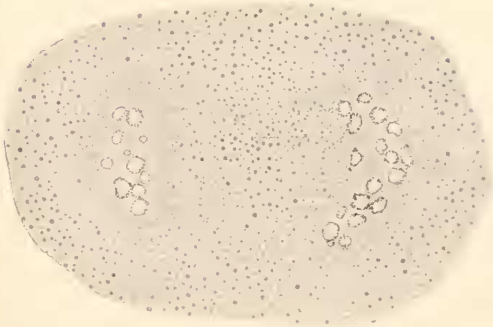

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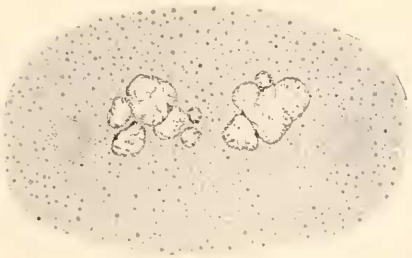

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Explanation of Plate II.

Pennaria. Figs. 9 and 10, × 1,300.

Clava leptostyla. Figs. 11 to 13, × 1,300; Fig. 14, × 350.

Fig. 9. Third or fourth cleavage spindle with "nuclear nest" passing on to it to form the equatorial plate, one vesicle broken up into chromosomes. *Pennaria.*

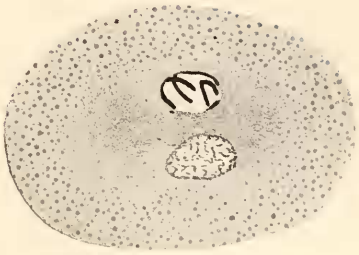
Fig. 10. Polar view of a cleavage spindle showing a "nuclear nest" as it is breaking up to form the equatorial plate, slightly later than the above, some of the chromosomes already free in the cytoplasm. *Pennaria.*

Fig. 11. Metaphase of first polar spindle of egg of *Clava leptostyla.*

Fig. 12. Anaphase of the second polar spindle of egg of *Clava leptostyla.*

Fig. 13. Female germ nucleus and polar bodies of egg of *Clava leptostyla.*

Fig. 14. Egg of *Clava leptostyla* showing the first cleavage spindle.



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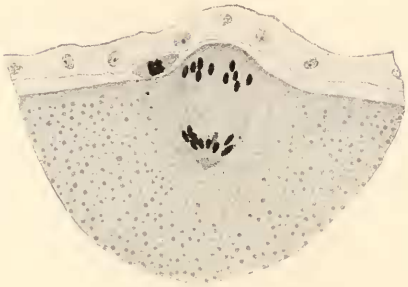

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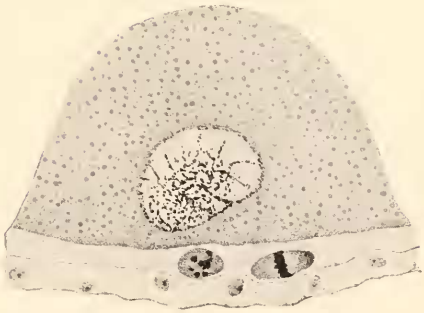

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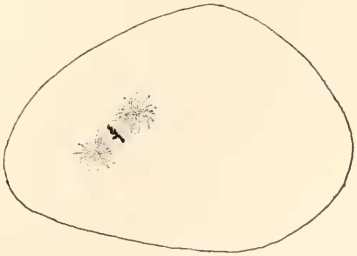

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Explanation of Plate III.

Clava leptostyla. Figs. 15 to 19, × 350.

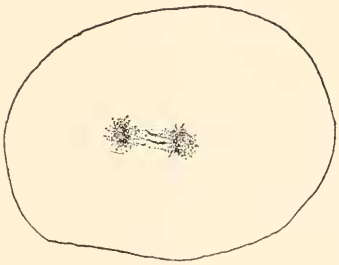
Fig. 15. Same, showing reconstruction of daughter nuclei by chromosomal vesicles.

Fig. 16. Two-cell stage of *Clava leptostyla* passing into the four-cell stage, second cleavage spindles showing.

Fig. 17. Four-cell stage of *Clava leptostyla* with resting nuclei.

Fig. 18. Four-cell stage of *Clava* passing into the eight-cell stage, spindles showing in two cells.

Fig. 19. Lateral view of eight-cell stage of *Clava* (five cells showing in section), some of the cells with spindles, some with resting nuclei.



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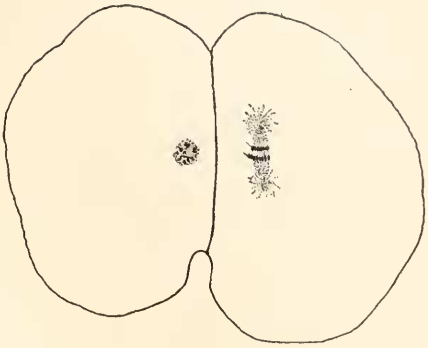

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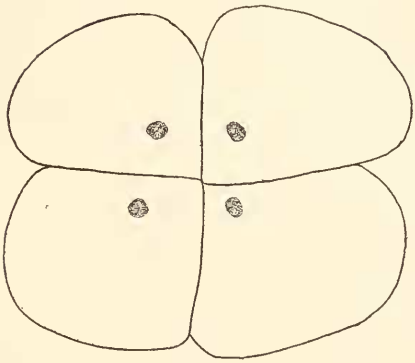

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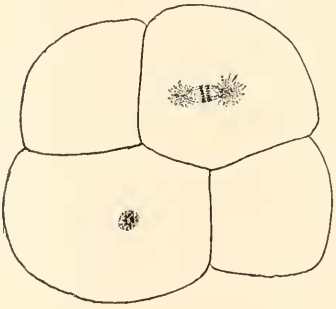

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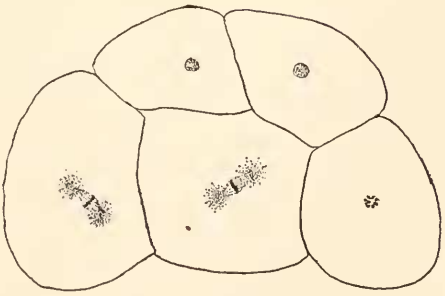

19

ON THE SEX OF HYBRID BIRDS.

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In a former paper[1] I have noted the difficulty of obtaining female hybrids from pigeons or doves of widely different parentage. Of the seven hybrid offspring of very distinct species then in hand, six were male. Since that time, through the courtesy of the Museum d'Histoire Naturelle in Paris and the Museum of Natural History in London, I have had the opportunity of examining a number of different hybrids in the family Phasianidæ and among them also I have found a remarkable predominance of males. In the following tabulations the sex of each hybrid, when known, and the parentage, is given, together with the date the individual was placed in the museum. It has been impossible to give the specific name always because a number of the specimens bore only the popular names. The letters placed after the year of accession indicate the respective locations of the specimen in question; thus, B = British Museum (Museum of Natural History); P = Museum d'Histoire Naturelle, Paris; C = Museum, University of Cincinnati.

Guinea-fowl × Chicken.

	Sex.	Date and Location.
Guinea-fowl × common fowl	= ?	1902 B.
Guinea-fowl × common fowl	= ♂	1899 B.
Pintade × Poule	= ?	1854 P.
Black Langshang Cock × Guinea-hen	= ♂	1903 C.
Black Langshang Cock × Guinea-hen	= ♂	1903 C.
Black Langshang Cock × Guinea-hen	= ♂	1903 C.
Black Langshang Cock × Guinea-hen	= ♂	1908 C.
Black Langshang Cock × Guinea-hen	= ♂	1909 C.

Thus, of eight guinea-chicken hybrids, the sex is known in six cases and it is invariably male.

[1] Guyer, M. F., "Spermatogenesis of Normal and of Hybrid Pigeons," Dissertation, University of Chicago, 1900. Also published as Bul. 22, University of Cincinnati, 1903.

PHEASANT × CHICKEN.

Chrysolophus pictus × Bantam fowl	= ♂	1890 B.
Phasianus colchicus × Game bantam	= ♂	1902 B.
Phasianus colchicus × Spanish fowl	= ?	1845 B.
Phasianus colchicus × Common fowl	= ♂	1884 B.
Phasianus colchicus × (Japanese long-tailed cock × common hen)	= ♂	1905 B.
Faison × Poule	= ♂	1851 P.
Faison × Poule	= ♂	1845 P.
Faison × Poule	= ♂	1836 P.
Faison × Poule	= ♂	1855 P.
Faison × Poule	= ♂	1813 P.
Faison × Poule	= ♂	1851 P.
Faison × Poule	= ♂	1851 P.
Faison × Poule	= ♂	1846 P.

It will be seen that of thirteen pheasant-chicken hybrids, the twelve of which the sex is recorded are all male.

PEAFOWL × CHICKEN.

Paon × Poule Cochinchinoise	= ♂	1907 P.
Paon × Poule Cochinchinoise	= ♂	1907 P

From the foregoing it will be observed that of the total of twenty-three hybrids from markedly different parentage (guinea × chicken, pheasant × chicken, and peafowl × chicken), each one of the twenty of which the sex is known is male.

PEAFOWL × PEAFOWL.

Pavo cristatus × *Pavo muticus*	= ♂	B.

PHEASANT × PHEASANT.

Chrysolophus pictus × *Phasianus reevesi*	= ♂	1887 B.
Hybrid *P. colchicus-reevesi* × *Gennæus nycthemerus*	= ?	B.
Chrysolophus pictus × *Phasianus colchicus*	= ♂	1855 B.
Hybrid *P. colchicus-reevesi* × *Gennæus nycthemerus*	= ♂	1904 B.
Gennæus horsfieldi × *Phasianus versicolor*	= ♂	1866 B.
Hybrid *P. reevesi-colchicus* × *Gennæus nycthemerus*	= ♂	1902 B.
Phasianus colchicus × *Gennæus nycthemerus*	= ♂	1902 B.
Hybrid *C. pictus-amherstiæ* × *Phasianus colchicus*	= ♂	1897 B.
Chrysolophus pictus × *Gennæus nycthemerus*	= ♂	1906 B.
Phasianus colchicus × *Chrysolophus pictus*	= ♂	1904 B.
Phasianus colchicus × *Gennæus melanotus*	= ♂	1865 B.
Phasianus colchicus × *Chrysolophus amherstiæ*	= ♂	1898 B.
Lophophorus impeyanus × *Euplocamus*[1] *melanotus*	= ?	1893 P.

[1] *Euplocamus* is a synonym of *Gennæus*.

[2] Presumably *C. pictus*.

Faisan doré[2] × Faisan commun[3]	= ♂	1842 P.
Faisan à collier[4] × Faisan argenté[5]	= ?	1886 P.
Faisan commun[3] × Faisan amherst[6]	= ?	1837 P.
Faisan doré[2] × Faisan ordinaire[3]	= ♀	1853 P.
Faisan commun[3] × Faisan argenté[5]	= ♂	1837 P.
Faisan commun[3] × Faisan argenté[5]	= ♂	1843 P.
Phasianus mongolicus × *Phasianus colchicus*	= ♂	1906 B.
Phasianus colchicus × *Phasianus reevesi*	= ♀	1904 B.
Phasianus colchicus × *Phasianus torquatus*	= ♂	1894 B.
Phasianus colchicus × *Phasianus reevesi*	= ♂	1894 B.
Chrysolophus amherstiæ × *Chrysolophus pictus*	= ♂	B.
Phasianus colchicus × *Phasianus reevesi*	= ♂	1897 B.
¾ *Chrysolophus amherstiæ* × ¼ *Chrysolophus pictus*	= ♂	1887 B.
Euplocamus[1] *swinhoii* × *Euplocamus nycthemerus*	= ♂	1882 P.
Euplocamus swinhoii × *Euplocamus nycthemerus*	= ♀	1875 P.
Euplocamus lineatus × *Euplocamus nycthemerus*	= ♂	1887 P.
Euplocamus lineatus × *Euplocamus nycthemerus*	= ♀	1878 P.
Euplocamus horsfieldi × *Euplocamus lineatus*	= ♂	1819 P.
Euplocamus horsfieldi × *Euplocamus lineatus*	= ♂	1869 P.
Faisan amherst[6] × Faisan doré[2]	= ?	1882 P.
Faisan amherst × Faisan doré	= ?	1902 P.
Faisan commun[3] × Faisan à collier[4]	= ?	1860 P.
Faisan commun × Faisan à collier	= ♂	1858 P.
Faisan commun × Faisan à collier	= ♂	1843 P.

Of a total of thirty-seven hybrid pheasants, nineteen have been from parents sufficiently widely separated to be ranked by systematists as separate genera or subgenera, and of these nineteen, fifteen were of known sex, namely, fourteen males and one female. Of the remaining eighteen there were twelve males, three females and three of which the sex was undetermined.

Thus of a grand total of sixty-one hybrids, the sex is known in fifty-one cases and among these there are only four females in all. Furthermore, three of these females were hybrids between species of the same genus, the other one, between species from genera not widely divergent. In hybrids between individuals of distantly related genera or between individuals from different subfamilies (*e. g.,* guinea × chicken) where the sex has been recorded it has been invariably male.

There are three possible sources of error in these data. In the

[3] Presumably *P. colchicus.*
[4] Presumably *P. torquatus.*
[5] Presumably *G. nycthemerus.*
[6] Presumably *C. amherstiæ.*

first place it is known that sterile females sometimes, although rarely, take on the male plumage, and it may be urged that there is no means of knowing certainly that the sex was determined beyond all doubt by opening the abdominal cavity and finding the testes. However, since the specimens had to be partially dissected before the skins could be mounted, it is reasonable to suppose that in the vast majority of cases the sex was thus accurately determined. The five guinea-chicken hybrids as well as the six dove and pigeon hybrids mentioned in my former paper were all dissected by me personally and consequently I am sure of their sex.

In the second place the objection may be raised that possibly the museums have preserved only the males, inasmuch as they make handsomer specimens and are not as similar in appearance as female pheasants. There is, of course, a possibility of this, especially in the case of hybrid pheasants from closely related species. Hybrids from widely different parents are so rare, however, that there is every probability that if there had been females they as well as the males would have been preserved. As a matter of fact, the few female pheasant hybrids that I have been able to find in museums are not similar in appearance nor do they resemble the males. As *hybrids* they are as interesting in every way as the males and it seems probable, therefore, that had there been more of them they would have been preserved. When due allowance is made for all errors the facts still indicate that there is a marked tendency for hybrids, especially those from widely separated parents, to be male.

Lastly, there is the remote possibility that there has been a greater mortality among the females in early life. In the few cases (guinea-chicken hybrids and various pigeon hybrids) of which I have data regarding the number of eggs laid and the history of the young, there is no evidence of such mortality.

It may be noted in passing that in the collections of the British Museum there is to be seen a hybrid between individuals of two different families, namely, a penelope (Family Cracidæ) and the common fowl (Family Phasianidæ). This hybrid resembles more the fowl than the penelope. Unfortunately the sex is not recorded.

In looking over the literature of the subject to see if anything had been recorded concerning the sex of hybrids outside the group Phasianidæ, I found that in general little attention had been paid to it. Some mention is made of the sex of hybrids in Suchetet's[1] voluminous work on hybrid birds. In speaking (p. cxvii) of hybrids and mongrels, he asserts that among the former he believes there are more males than females, and he cites various authorities in substantiation of his belief. Thus, according to data collected by Buffon, there are more male than female mules and Buffon asserts, furthermore, that among hybrid birds the number of males exceeds very much that of females. Suchetet cites the following figures from Buffon: the proportion of males to females in hybrids between the he-goat and the ewe are 7 to 2; between the dog and the wolf, 3 to 1; between the goldfinch and the canary, 16 to 3. Suchetet cites still further examples from other authorities, but he seems not to have gone over his own extensive notes on hybrids with this question of sex in mind. For example, on pages cxxi–cxxxiv he gives a statement in tabular form of data collected from some eighty-five public and private museums concerning in all 234[2] specimens of hybrids between wild birds (*i. e.,* not domesticated) or of forms reputed to be such hybrids. Since in many cases the sex of these hybrids has been given, I have gone through the tables and arranged the birds according to sex as far as it is indicated, with the following results:

Of hybrids between species bearing the same generic name there are in all 124, of which 72 were male, 18 female and 34 of undetermined sex. The remaining 110 hybrids were between individuals bearing different generic names and of these 74 were male, 13 were female and 23 were of undetermined sex. Thus it will be seen that the males far outnumber the females in each case. Furthermore, this would remain true in the proportion of about 3 to 2, even should it be counted that all those of *undetermined* sex were female!

In his later amplifications of this list he discusses (p. 507) 48

[1] Suchetet, André, "Des Hybrides à L'Etat Sauvage; Oiseaux," Vol. I., 1896, Lille. A large volume of over 1,000 pages.

[2] Suchetet states the total as 236 but he has made an error of 2 in his addition on page cxxxii.

additional hybrids between *Tetrao tetrix* and *Tetrao urogallus,* of which 40 are male and 8 female. Again, page 573, he lists 20 hybrids of *Lagopus albus* and *Tetrao tetrix,* of which 13 are male and seven are female.

As to the general bearing of these facts upon any one of the numerous theories of sex-determination, the writer does not feel disposed to dogmatize, although certain suggestions present themselves. For a general and unbiased statement of our present knowledge regarding the question of sex-determination, the reader may consult the recent publications of Thomson[1] or of Morgan.[2]

Both of these writers agree that when all the evidence is considered it does not seem improbable that the conditions which regulate the development of sex may be different in different kinds of animals. Regarding the sex-determining influence of nutrition and temperature, either directly on the developing organism or through its parents, Thomson points out that while the evidence in any given case is inconclusive, still when all the cases are taken together, " they have a certain cumulative suggestiveness which would warrant further experiment—particularly as regards the lower animals and the indirect influence on offspring through the parents " (1908, p. 490).

In general, where the experiments tend to show that nutrition is a factor after the period of fertilization, it has been the production of females that was supposedly favored by such increased nutrition; the question being apparently one of increased constructive metabolism. It would follow that anything tending to retard or hold at a low ebb the constructive phases of metabolism, especially during early embryogeny, would be inimical to the production of females. Now in the case of hybrids, and particularly those from widely separated parents, there would in all probability be more or less default in the metabolic processes because of the incompatibilities which must necessarily exist between two germ-plasms so dissimilar. It seems not improbable, therefore, that this might be the determining factor in the production of an excess of males in the case of such hybrids.

[1] Thomson, J. Arthur, " Heredity," London, 1908.

[2] Morgan, T. H., " Experimental Zoology," New York and London, 1907.

THE FEMALE CHROMOSOME GROUPS IN SYROMASTES AND PYRROCHORIS.

EDMUND B. WILSON.

The conditions seen in *Syromastes marginatus* L. are of interest on account of the light that they throw on those observed by Morgan in Phylloxera, as reported by him at the December meeting of the American Society of Zoölogists, and recently published in the issue of *Science* for Feb. 5, 1909. In both forms the "accessory" chromosome is not a single but a double body, and the female chromosome-groups contain two more chromosomes than the male.

A reëxamination of the spermatogenesis of *Syromastes*[1] led to a confirmation of Gross's result that the spermatogonial number is even (22), and that the "accessory" chromosome is formed by the union of two chromosomes that are separate in the spermatogonia. This double element divides equationally in the first spermatocyte-division but passes undivided to one pole in the second, so that half the spermatozoa receive two more chromosomes (12) than the other half (10). This led me to the inference that the female somatic groups should have two more chromosomes than the male—*i. e.*, 24 instead of 22, as had been described by Gross. I had at that time no female material, but through the kindness of Professor Boveri have since obtained an abundant supply of the ovaries. Examination of this material demonstrated the correctness of my earlier inference. A considerable number of ovaries have been sectioned, many of which contain numerous and very fine division-figures, showing the chromosomes with great clearness. Whenever a good view of the equatorial plate can be obtained, 24 chromosomes are unmistakably seen to be present, as is clearly shown in photographs. Two figures (Fig. 1, *c*, *d*) are appended, both of which were drawn upon enlarged photographs by the method described in my fourth "Study." *Syromastes* is an exceptionally favorable form

[1] "Studies on Chromosomes, IV.," *Journ. Exp. Zool.*, VI., 1, 1909.

for study, and the diagrammatic clearness with which the chromosomes appear in many of the sections precludes, I think, the possibility of error in respect to the number.

In my description of the male groups I emphasized the fact that the two components of the "accessory" are of slightly un-

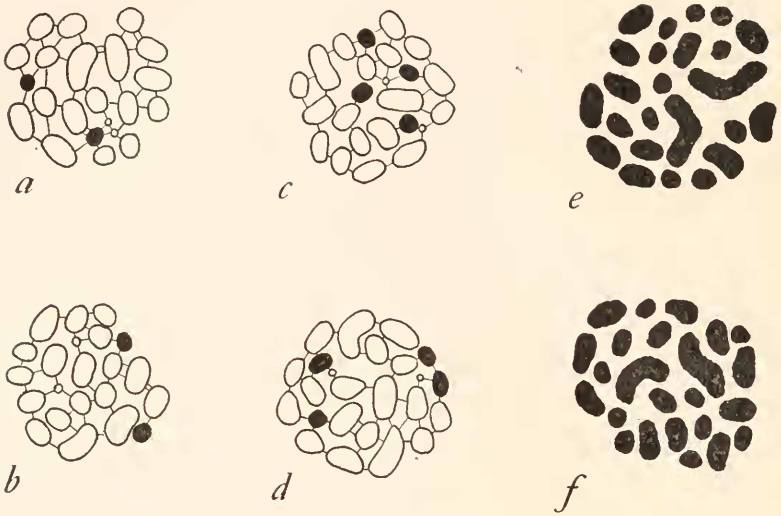

FIG. 1. *a, b, Syromastes marginatus* L., spermatogonial chromosome-groups; *c, d,* ovarian groups of the same; *e, f, Pyrrochoris apterus* L., ovarian chromosome-groups.

equal size (as is shown in the photographs accompanying the paper), and that they are recognizable in the spermatogonial groups as two separate chromosomes which are the second and third smallest of all the chromosomes. In the female groups each of these chromosomes is represented by a corresponding pair (black in the figures). In most of the ovarian groups the smaller two are readily recognizable, and in some cases, though not always, this is also true of the larger pair. The numerical and size-relations are such as to show that after maturation the egg must contain one member of each of these pairs. Though nothing is directly known of the maturation-process in the female, it may be inferred with probability that in synapsis the two larger and the two smaller of these pairs unite to form two corresponding bivalents, which may be designated as *aa* and *bb* (*II* and *ii*,

in the terminology of my former paper). By the subsequent disjunction of each of these pairs the mature egg receives *a* and *b* in addition to 10 other chromosomes. Fertilization of the egg by a spermatozoön containing the "accessory" $(a + b)$ will therefore give the characteristic female group $(a, b, a, b + 20)$, while fertilization by one that lacks $a + b$ will give the male group $(a, b + 20)$. For the sake of comparison two of the spermatogonial groups are reproduced in Fig. 1, *a, b*. In the figures of both sexes the chromosomes identified as *a* and *b* are made black. The essential relations in both sexes are shown in the diagram,

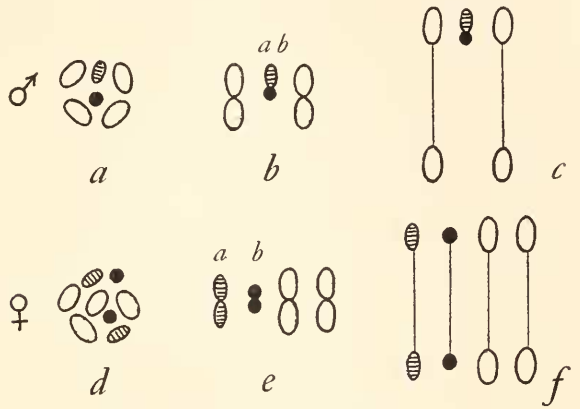

FIG. 2. Diagrams of maturation in *Syromastes*. *a*, spermatogonial chromosomes (actual number 22); *b, c*, spermatocyte-division; *d*, ovarian chromosomes (actual number 24); *e, f*, maturation division (inferred).

Fig. 2, in which *a* is cross-barred, *b* black, and the other chromosomes (of which only four are represented) are in outline.

It is evident that, except for the different total number of chromosomes in the two species, these phenomena are essentially similar to those seen in *Phylloxera fallax*, which likewise has two "accessories" and two more chromosomes in the female somatic groups (12) than in the male (10). In *Phylloxera caryæcaulis* the two "accessories" are of unequal size, as in *Syromastes*, but the phenomena are complicated by the fact that these two chromosomes are often united in the somatic groups of the male, and are apparently always thus united in those of the female. In the male they are always united at the time of the spermatocyte-

divisions to form the "accessory," which is in reality double, like that of *Syromastes,* and sometimes separates into its two components as it moves to the pole. Morgan therefore concludes that the true numbers of the chromosomes in the two sexes of this species are eight and six respectively, though the female seems to show but six and the male either five or six (according as the two "accessories" are united or separate).

The point to which I would call attention is the similarity between *Phylloxera caryæcaulis* and *Syromastes* in respect to the mode of synapsis. In the spermatogenesis of both forms the two unequal "accessories" unite in synapsis (if the process can properly be so called) to form the bivalent *ab,* consisting of two unequal components, which passes into the female-producing spermatozoa. In the maturation of the male-producing egg of *Phylloxera,* however (and apparently the same must be true of the sexual eggs), a different process takes place, the two larger and the two smaller components uniting to form the bivalents *aa* and *bb,* again exactly as there is reason to conclude in the case of the egg of *Syromastes.* In *Phylloxera,* as Morgan points out, this involves a redistribution of the four chromosomes, since in the somatic groups they are united to form *ab* and *ab,* but recombine at the maturation period to form *aa* and *bb.* This remarkable redistribution, I think, loses much of its anomalous character on comparison with the facts in *Syromastes,* where *a* and *b* are always separate in the somatic groups.

These facts, together with those determined by Payne in *Fitchia* and other forms (now in press in this journal) and my own earlier ones on *Thyanta,*[1] which shows essentially the same conditions as in *Fitchia,* lead me to a somewhat different interpretation of the "accessory" chromosome in *Syromastes* from that given in my fourth "Study." In that paper I adopted the conclusion that the two components of the bivalent "accessory" were identical respectively with the large and small "idiochromosomes" of such forms as *Metapodius* or *Lygæus.* I did not then see that all the facts are equally consistent with the view that these two components, *taken together,* represent the single odd

[1] Reported at the meeting of the American Society of Zoölogists in December, 1906, but still unpublished.

chromosome of *Anasa, Protenor* and other similar forms, and that the small idiochromosome has disappeared. Payne discovered that in the reduvioids, where a single small idiochromosome or "Y-element"[2] is always present, the large idiochromosome is in some species a single chromosome (*Diplocodus*), in others is represented by two (*Fitchia, Conorhinus*) or three chromosomes (*Prionidus*), and in the galgulid genus *Gelastocoris* (*Galgulus*) by four chromosomes, which behave in maturation as a single unit (X-element) that is obviously comparable to a single large idiochromosome in its relation to sex-production. Payne concludes, with great probability, that the double or multiple X-element in these forms has arisen by the separation of an originally single large idiochromosome (such as still exists in related species) into two or more components. In *Conorhinus,* where the X-element is double, the two components are unequal in size, and by the disappearance of the Y-element a condition would arise closely similar to that seen in *Syromastes.*

Whether such has been the actual mode or origin in *Syromastes* or not, it seems probable that here too the double "accessory" was originally a single chromosome that has separated into two parts, which still act as a unit in the maturation divisions and retain the same relation to sex-production as the original one. *Phylloxera caryæcaulis* may plausibly be regarded as in process of transition from the condition in which a single "accessory" chromosome is present (as appears to be the case in the aphids) to one in which it has separated into two parts, as in *Syromastes.*

I will add a brief account of the female groups in *Pyrrochoris apterus* L., material for which has also been obtained through Professor Boveri. A reëxamination of the male groups (Wilson, Study IV.) showed the spermatogonial number to be 23, including a single unpaired idiochromosome ("accessory" chromosome) which is at once recognizable from the fact that it is nearly twice the size of any of the other chromosomes. This passes into half the spermatozoa, which receive 12 chromosomes, while the others receive but 11, as was originally described by Henking. The

[2] In a recent general discussion I have used the terms "X-element" and "Y-element" to designate respectively the large and small idiochromosomes, or their homologues, whether they consist of a single chromosome or of more than one. See *Science,* XXIX., 732, January 8, 1909.

female groups clearly show 24 chromosomes, of which two are of the same relative size as the unpaired one of the male. Two of the ovarian groups, typical of many others observed, are shown in Fig. 1, *e, f*. These facts show that *Pyrrochoris* conforms to the ordinary type in which the male has an odd chromosome, as in *Anasa, Protenor, Alydus, Largus* and many others.

COLUMBIA UNIVERSITY,
January 23, 1909.

OBSERVATIONS ON THE GERM CELLS OF HYDRA.

GEO. W. TANNREUTHER.

The germ cells of hydra, which pass through stages comparable to those of higher animals, possess some peculiarities that have not been previously mentioned. Some investigators advocate the specificity of the germ cells, while others claim that the sex cells are the immediate derivatives of the ordinary interstitial cells.

Kleinenberg (4), Hertwig (3), Brauer (1) and others consider the sex cells as interstitial in origin and state that the egg first becomes recognizable after the ovary has begun to develop. Downing (2) claims that there is continuity of germ plasm in the sense of a specific line of germ cells, that the egg (*Hydra fusca*) is always present even before the interstitial cells begin to form the ovary and that the egg may grow rapidly and take the initiative in its formation. He furthermore believes that the egg is recognizable as such in the adult hydra and in general that in some stage in the embryonic development certain cells are stamped with sex characters, so that they and their progeny form the sex cells distinct throughout the life of the individual.

A careful examination of sections from *Hydra* sp.? (Brauer) gives pretty conclusive evidence that not only the egg but the sperm as well is interstitial in origin. There can be no question in case of the sperm, as the different stages in development can readily be traced from the interstitial cells to the mature sperm. Furthermore, the progenitors of the spermatozoa have no special characters by which they can be recognized as germ cells. The cells that give rise to the eggs are interstitial in position and can be distinguished in the adult hydra from the ordinary interstitial cells by their large nucleus, nucleolus and abundance of chromatin, even before the growth of the ovary begins, as Downing states. This is especially true during the breeding season. If these sex cells could be distinguished during the budding season as well, it would at least suggest specificity of the germ cells.

In the dicœcious form *Hydra* sp.? (Brauer) the egg cells can-

not be distinguished from the ordinary interstitial cells except during the period of sexual reproduction. Fig. 1, *a* and *b,* represents two adjacent eggs at the stage of development when they first become recognizable. They have begun to enlarge and can readily be distinguished from the adjoining cells by their size and vacuoles next the nucleus. The above figure was taken from an adult hydra in which six eggs were present in different stages of development, ranging from the interstitial cells to the undisputed egg. These eggs are isolated or found in groups. When two or more are found adjacent the cell walls become dissolved and one persists as the developing egg (Tannreuther). In a few instances observed two of these adjacent cells persisted and gave rise to two mature eggs. These cases, however, are extremely rare in proportion to the number of eggs produced.

The above results do not warrant the view that there is continuity of germ plasm in *Hydra.* Until sex cells distinct from the somatic can be traced through successive generations, we have no positive evidence of such a continuity.

The different generations of the sex cells of *Hydra* are distinct and can readily be recognized. In the formation of the egg there is a distinct growth period. Reduction occurs at the end of the growth period just before the first polar body is formed. The polar bodies remain attached after cleavage has begun, by means of a single cytoplasmic thread. The first polar body is larger than the second.

The spermaries are composed of an indefinite number of cysts. The individual cysts originate from a single or several interstitial cells. Fig. 2 represents a longitudinal section of a single cyst containing spermatogonia, which have originated from a single interstitial cell. After the spermatogonia have divided a few times (the number of divisions varies in different individuals) those found at the distal end of the cyst become transformed into the spermatocytes of the first generation without growth. In Fig. 3 the spermatocytes of the first generation are recognized by their dense chromatin mass. Different stages of development are found in a single cyst, ranging from the spermatocytes of the first generation to mature sperm, as shown in Fig. 4. The successive zones of the developing sperm are distinct. The nuclei

at the extreme proximal end of the cyst cease dividing and become the nuclei of new interstitial cells after the spermaries have disappeared.

My observations on the nuclear changes in the different stages of development confirm those of previous investigators. Reduction occurs after the last spermatogonial division just before the spermatocytes of the first generation divide. In the division of the nuclei of the spermatocytes of the first and second generations, the cell wall remains intact and produces a multinucleate cell (Figs. 5–7), and give rise to four sperm within a common

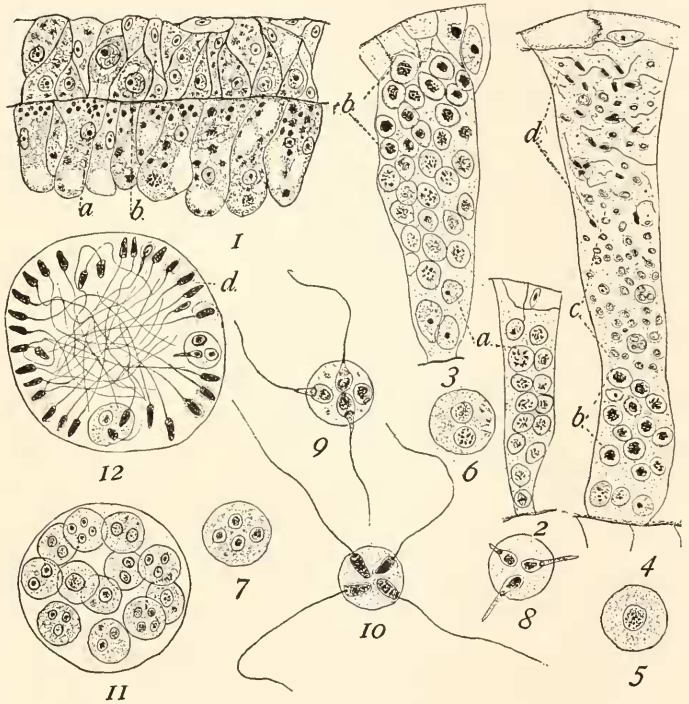

Fig. 1. Longitudinal section showing two developing eggs. *a* and *b*, eggs.

Fig. 2. Longitudinal section of a single cyst.

Fig. 3. Section of cyst little later than preceding. *a*, spermatogonia; *b*, spermatocytes of the first generation.

Fig. 4. Longitudinal section of cyst showing maximum development. *c*, spermatocytes of the first and second generations; *d*, mature sperm.

Figs. 5–7. Formation of spermatids within common vesicle.

Figs. 8–10. Developing sperm. Figs. 5–10, from living cells.

Figs. 11 and 12. Cysts removed from living spermary.

vesicle. The vesicular wall is very thin and the four sperm pass through it when mature and become free within the distal end of the cyst (Fig. 4). The mature sperm are very active within the cyst and finally escape to the exterior through a small temporary opening of the spermary. After the mature sperm have all escaped, the opening closes until more sperm mature. When the spermaries are extremely large, this process continues from forty to fifty hours.

In order to show the four sperm within a common vesicle, it is necessary to dissect the living spermary apart, as the sperm always escape from the common vesicle before passing to the exterior. The individual cysts when separated become more spherical (Figs. 11 and 12), and the different stage of development in the living sperm can easily be distinguished.

Kleinenberg in his description of the sperm in *Hydra viridis* definitely states that the sperm are formed from interstitial cells that have divided a number of times; ultimately the nucleus of the cell (the spermatocyte of the first order) disintegrates while the cell substance becomes granular, and in place of the nucleus there appears from one to four refractive bolies, which give rise to the sperm. The refractive bodies referred to beyond doubt result from the two divisions of the nucleus, which he thought disintegrated. The four nuclei (spermatids) within the common vesicle do have the appearance of refractive bodies, especially in the living material.

Korotneff (5) gave similar results. He states that the sperm form directly from the nuclei of a multinucleate mother cell. Downing (2) does not mention this interesting phenomenon, which is found in *Hydra viridis* and *Hydra* sp.? (Brauer).

The mature sperm possess extreme vitality and may remain active from one to three days after escaping from the spermary. The mature sperm of any individual spermary possess about the same degree of fertility. No degenerating spermatogonia were found.

In the monœcious form *Hydra viridis* the spermaries become mature before the ovaries. Occasionally sperm and ova on one individual would ripen at the same time, making self-fertilization possible. In order to prove that self-fertilization did occur, indi-

viduals with both spermaries and ovaries at the same stage of development were isolated and placed in distilled water for one hour, in order to kill any mature sperm that might adhere from other individuals. The *Hydra* were then placed separately in water free from sperm. The spermaries and ovaries matured and self-fertilization took place. The cleavage was normal.

Summary.

1. *Hydra* sp.? (Brauer) does not show continuity of the germ plasm.

2. The eggs can be distinguished from the interstitial cells in the adult *Hydra* before the ovary is formed. This is especially true during the breeding season.

3. The different generations in the formation of the germ cells are comparable to those of higher animals.

4. In the division of the spermatocytes of the first and second generations the cell wall remains intact and the four spermatids are formed within a common vesicle, each producing a mature sperm.

5. Self-fertilization occurs in *Hydra viridis*.

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BUDDING IN HYDRA.

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Budding in hydra has received the attention of only a few investigators. Our principal information as to the origin of the bud is derived from Lang's[1] account. He describes the bud as beginning by an increase in volume and division of the interstitial cells. After the ectoderm becomes thickened, the mesoglea disappears and the cells pass from the ectoderm into the endoderm. This process continues until the ectoderm becomes reduced to its normal thickness. The mesoglea then reforms and a cavity appears in the thickened endoderm, which becomes the enteron of the new individual.

The main object of the present paper will be to give a brief account of the origin and development of the buds in hydra, more especially their manner of growth and what cells contribute most to their rapid formation.

The species studied, *Hydra viridis* and *Hydra* sp.? (Brauer) differ somewhat from Lang's account. The mesoglea does not disappear and the ectodermal cells do not pass into the endoderm. The bud, however, begins by an increase in volume and division of the interstitial cells. After they have increased once or twice in volume, as shown in Fig. 1, there is a slight outbulging of the ectoderm, which is scarcely perceptible. Fig. 2 represents the condition of the interstitial, ectodermal and endodermal cells in the origin of the bud as they appear more highly magnified. A few mitotic figures are visible. No amitotic divisions were observed. The endodermal cells contain numerous food particles, which may pass intact through the mesoglea into the ectoderm. The cells directly concerned in the formation of the bud are well supplied with food, while the remaining cells of the parent hydra show a scarcity. Many of the endodermal cells in the distal half of the hydra have a glandular appearance and are most active in

[1] Lang, Albert, "Uber die Knospung bei *Hydra* und einigen Hydropolypen," *Z. wiss. Zool.*, Vol. 54, 1892.

the secretion of the digestive fluid, which aids in the breaking up of the food in the enteron, while those endodermal cells in the region of growth, "the formation of buds and sexual organs," are the most active in ingesting the partly digested food from the enteron and preparing it for diffusion into the ectodermal cells.

The ectoderm and endoderm in the early formation of the bud, which are quite uniform, soon become differentiated into two dis-

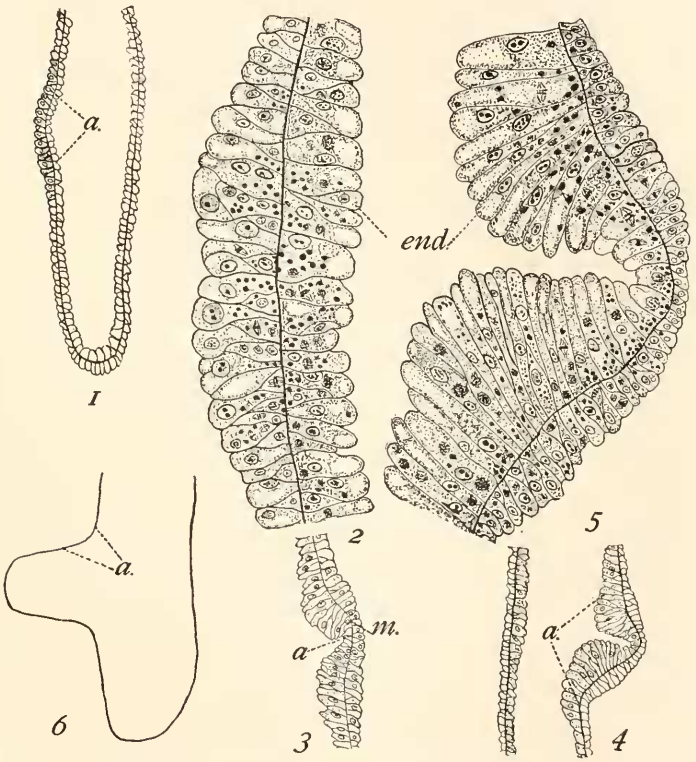

FIG. 1. Longitudinal section of hydra showing the origin of bud at *a*.

FIG. 2. Part of preceding figure at *a*. × 80.

FIG. 3. Longitudinal section through wall of hydra showing curvature of mesoglea and beginning of enteron in forming bud. *m*, mesoglea; *a*, enteron.

FIG. 4. Stage of development a little later than preceding. *a*, the point of junction between parent and bud.

FIG. 5. A portion of preceding figure at *a*, which includes the entire area in the formation of bud. *end.*, endoderm. × 90.

FIG. 6. Diagram of a longitudinal section of parent hydra with bud. *a*, active growing region in formation of bud.

tinct regions. The cells more centrally placed (Fig. 3, *a*), which correspond to the distal end of bud, become inactive, while those on either side of the central region continue active, divide rapidly and contribute almost entirely to the rapid growth of the bud. There is a slight curvature in the mesoglea (Fig. 3, *m*), and the enteron of the new individual becomes apparent. In a stage little later than the preceding (Fig. 4), the relation of bud to parent and the condition of cells is more plainly represented. Fig. 5, a portion of Fig. 4 at *a*, represents the entire area that contributes directly to the growth of the bud. The cells at the apex of the forming bud are small. Those on either side are larger and more active in the process of division and growth. Their contents is very similar, showing an abundance of food material and cytoplasmic granules.

The ectoderm and endoderm at the junction of the parent and bud (Fig. 7) divide very rapidly and become the most active growing region in the production of the new individual. The remaining cells of the bud seldom divide. The formation of the tentacles is similar to that of the buds. The cells corresponding to their basal ends take the most active part in their growth. The mouth is formed at the distal end of the bud by a breaking through of the ectoderm.

The rate of growth of the bud is determined by the amount of food present. In an active feeding hydra, the buds are often completely formed in thirty to forty hours. While in those hydras with a moderate supply of food the buds grow very slowly and may require four or five weeks or even more time for their complete development. In the latter instance after the buds are nearly formed, they will be absorbed in the absence of food. In the process of absorption the cell walls of the bud become imperceptible and the cell contents presents the appearance of a complete syncytium. When the buds are nearly absorbed, if the hydra is again supplied with food, the buds very seldom reform. In a few instances the buds were neither absorbed nor reformed, but remained attached to the parent as permanent individuals. Tentacles were formed later.

When the buds have reached their complete development the ectodermal cells at the proximal end undergo a rapid change

(Fig. 8, *ect*). They become more narrow, elongated and present the appearance of glandular cells. They have the power to

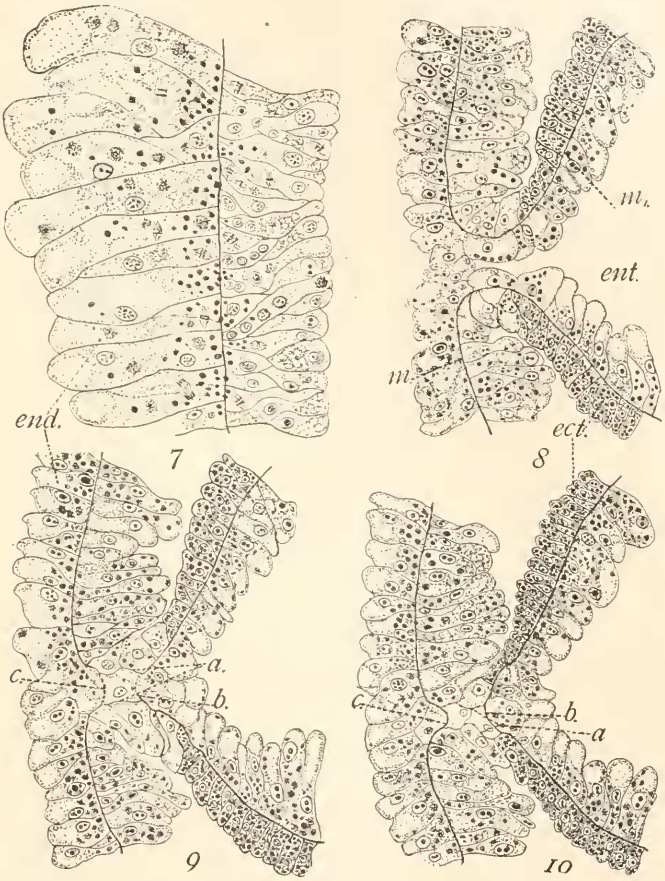

FIG. 7. Longitudinal section at junction of parent hydra and bud, taken at *a* in Fig. 6. *end.*, endoderm. × 100.

FIG. 8. Longitudinal section through base of mature bud and wall of parent hydra. m_1, mesoglea of bud; *m*, mesoglea of parent; *ect.*, glandular ectoderm; *ent.*, enteron of bud.

FIG. 9. Longitudinal section showing change of mesoglea in separation of parent and bud at *a*, and formation of new mesoglea at *b* and *c*. The endodermal cells at proximal end of bud have united. *a*, mesoglea between bud and parent becoming thinner; *b*, new mesoglea of bud; *c*, new mesoglea of parent.

FIG. 10. Longitudinal section showing completion of mesoglea at *b* and *c* and persistence of old mesoglea at *a*.

secrete a sticky substance before the bud becomes separated from the parent.

The first step in the process of separation of bud from parent occurs in the mesoglea. Fig. 8 represents the condition of mesoglea and cells before separation begins. The mesoglea, which connects the bud with parent, is uniform throughout. But almost immediately it becomes thinner and thinner until it is indistinguishable from the ordinary cell wall (Fig. 9, *a*). The enteron leading from the parent to the bud becomes discontinuous by the union of the endodermal cells. New mesoglea is now formed at the extreme basal end of the bud (Fig. 9, *b*) and at the point of former union with the parent at *c*. The mesoglea, which is more of a gelatinous nature, increases in thickness by means of secretion from the endodermal cells and soon reaches its normal condition (Fig. 10, *b* and *c*). After the formation of the mesoglea is complete, the bud remains attached to the parent by a few ectodermal and endodermal cells, as shown in Fig. 10. The former connecting mesoglea is represented by the dotted lines at *a*. The cells between the dotted lines, which are endodermal, become external to the newly formed mesoglea and take the position of ectoderm. Whether these cells persist or not and function as ectoderm is difficult to say, as there is no possible means of following them in the process of separation of bud from parent.

Summary.

1. The initial step in the formation of bud in hydra is found in the interstitial cells.

2. The most active region of growth in the formation of the bud is found at the junction of the forming bud and parent, where the cells divide very rapidly and contribute almost entirely to its growth.

3. When the bud is nearly formed the ectodermal cells in the basal region become transformed into granular glandular cells, which later secrete a glutinous substance for attachment of hydra.

4. The rate of growth in buds is controlled by the amount of food present. Starvation after buds are nearly complete often causes their complete absorption by parent hydra.

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THE NUCLEOLI IN THE SPERMATOCYTES AND GERMINAL VESICLES OF EUSCHISTUS VARIOLARIUS.

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I. In the resting first spermatocyte of many insects there appears a spherical, chromatic body which invariably stains as intensely as the chromosomes.

McClung ('02) was the first investigator to draw special attention to this structure by his suggestion that it may function as a sex determinant.

A majority of the cytologists who have studied this structure interpret it as one of the chromosomes which persists through the rest stage of the first spermatocyte, and they claim that its presence or absence in a spermatozoön is the determining factor of sex, McClung assuming that its presence in a spermatozoön causes the fertilized egg to produce a male, whereas Stevens, Wilson and others affirm that its presence in a spermatozoön causes the fertilized egg to produce a female, and in those cases where two (a large and a small) chromatin nucleoli are present, the larger is the female-determining, and the smaller, the male-determining factor.

Identifying the chromatin nucleolus as a chromosome enlarges at once the sphere of the problem, involving this structure in the maze of hypotheses and theories associated with the chromosomes. This identification of the chromatin nucleolus as a chromosome is of great importance, because it presumably demonstrates that a definite chromosome retains its individuality throughout the rest stage, and it presents very strong evidence for the theory of the individuality and continuity of the chromosomes — the theory which is the corner-stone of all hypotheses involving the

claim that the chromosomes are the cause rather than the expression of cell activities.

We believe that the evidence we find in *Euschistus*, as well as in *Anasa* ('07), is distinctly opposed to the interpretation that the chromatin nucleolus of the resting first spermatocyte is a phase of one or more of the chromosomes, but we shall reserve the publication of this evidence in *Euschistus* until we can control it by a more complete comparison with other forms. In the present paper we shall limit ourselves to the relatively simple question, is the chromatin nucleolus a structure associated with the male cell only, as claimed by McClung, or is it, as claimed by Wilson, a chromosome received from the egg, which during the rest stage appears in the form of a chromatin nucleolus?

Two chromatin nucleoli unequal in size have been demonstrated by Montgomery ('98, '01, '06) in the first spermatocytes of several varieties of *Euschistus*, including *Euschistus variolarius*, and Wilson ('05) has demonstrated these structures in other Hemiptera, naming them chromosome nucleoli, because he believes them to be chromosomes. In describing them in *Cœnus* and *Lygæus* he says: "Throughout the whole of the growth period in *Cœnus*, and from stage *e* onward in *Lygæus*, at least one of the idiochromosomes can always be distinguished as a compact, spheroidal, intensely staining chromosome nucleolus and frequently both idiochromosomes are distinguishable in this form in all of these stages. . . . It is clear beyond all question that at least the large idiochromosome may retain its identity throughout the whole growth period, with the small idiochromosome the case is not so strong" (p. 389). Of *Brochymena* he says: "There can be no doubt that when only one chromosome nucleolus is present it is to be considered as a bivalent body arising by the fusion or synapsis of the two idiochromosomes" (p. 392).

In the resting first spermatocyte of *Euschistus variolarius* the chromatin nucleolus is the most deeply stained and conspicuous structure in the cell (Photos 1–10). As a rule, only one is present, but quite frequently there are two, sometimes equal, but in most cases, very unequal in size; exceptionally we have found three, or even four, nucleoli in the same nucleus.

We roughly estimated the number of the nuclei with one or

more chromatin nucleoli, by counting 625 cells in one testis, and of these cells 591 showed one nucleolus, 27 two nucleoli, and 7 three nucleoli. These 625 cells represented only a small area of the preparation and we did not repeat the experiment on another testis — the estimate, therefore, offers merely an indication that in a large majority of cases only one chromatin nucleolus is present.

By a comparative study of the spermatocytes and oöcytes of the same form we ought to find an answer to the question, whether the chromatin nucleolus is associated with the male cell only, or whether it has its origin in the oöcyte.

As far as we are aware, the germinal vesicles shown in Plates I., II. and III. are the first that have been demonstrated in any of the Hemiptera heteroptera. A great deal has been written about the spermotocytes and important generalizations have been drawn from the behavior of the chromatin nucleolus in these male cells, but not one word has been said about the corresponding, very important stages in the female, although we have been confidently assured that the chromatin nucleolus in the male has been derived from the egg. If this is true, it would seem at least logical to expect to find some evidence of its presence in the egg at the stage of development corresponding to the stage in which it is so conspicuous in the male cell.

Even if we assume for the sake of argument that the chromatin nucleolus is a chromosome, we still do not avoid the logical expectation of finding it in the oöcytes, for if one of the chromosomes of the spermatocyte possesses the distinguishing trait of persisting through the rest stage in the form of a chromosome nucleolus, and if that chromosome is a chromosome contributed to the male cells from the mother, we should expect to see its distinguishing characteristic manifested in even a more marked degree in the oöcyte. And further, as the chromosome nucleolus of the spermatocyte is claimed to be only one of a pair of chromosomes — its mate being contributed by the egg at the time of fertilization — we should expect to find in the resting oöcyte a pair of chromosomes represented by two univalent, or one bivalent chromosome nucleolus.

In the case of *Euschistus* we are told that the larger of the two chromatin nucleoli of the spermatocyte is the homologue of the

accessory chromosome of other forms, and if this interpretation is correct we may expect to find a large bivalent or two univalent chromatin nucleoli in the growing oöcytes.

II. We find the chromatin nucleolus of the spermatocyte persisting through the entire growth period, Photos 1 to 10. All the resting spermatocytes shown in these photos, are from testes which contain many first spermatocyte metaphases, in which the idiochromosomes show the typical inequality in size. We have selected preparations of the resting spermatocytes showing variations in the relative size of the two chromatin nucleoli (when present) in order to demonstrate their frequent lack of conformity to the size relations of the idiochromosomes. Photo 10 shows a nucleolus persisting until the chromosomes are formed and we have several photographs demonstrating its presence to a still later stage, where the seven bivalents can be counted, but we shall reserve the discussion of these later stages for a subsequent paper.[1]

As the chromatin nucleoli of the first spermatocyte of *Euschistus* persist through the entire growth period, we certainly have a right to expect to find in the egg the equivalent of the large chromatin nucleolus during these same stages, if as asserted it owes its origin to the egg. Again, if the so-called male sex-determinant (the small chromatin nucleolus) persists through the growth stages in the male cell, there seems to be no clear reason why the so-called female sex-determinant (the larger chromatin nucleolus) should not persist through the growth period of the oöcyte.

A study of the oöcytes shown in Photos 11 to 30 demonstrates that in none of these cells can be found a structure resembling in any way the chromatin nucleolus shown in the spermatocytes of Photos 1 to 10. This is clearly demonstrated in the spermatocytes and oöcytes of Plate I. There is no structure in the oöcytes of Photos 11 to 19 which resembles in the least the chromatin nucleolus of the spermatocytes of Photos 1 to 10. The only nucleolus in the oöcytes is a relatively large achromatic structure, first clearly demonstrated in the older oöcytes. It is

[1] We have a number of photographs showing the transition stages between Photos 9 and 10, but lack of space prevents their reproduction on these plates.

clearly seen in the germinal vesicles of Photos 18 and 19, Plate I., and in the germinal vesicles of Photos 20 to 25, Plate II., and in Photos 26 to 30, Plate III. All these preparations show, that as a rule its position is peripheral and this is also true of the principal nucleolus of the germinal vesicles of *Allolobophora* (Photo 31). The absence of a chromatin nucleolus is conspicuous not only in the germinal vesicles of *Euschistus* but in the young oöcytes as well. Compare the young oöcytes of Photos 11 and 12 with the young spermatocytes of Photos 1 to 5.

In none of the oöcytes, from the earliest to the latest stages do we find a chromatin nucleolus, and its absence in the germinal vesicles of *Euschistus* is made more conspicuous by the fact that a chromatin nucleolus is invariably present in the germinal vesicles of *Allolobophora*. If we could find such a structure in the germinal vesicles of *Euschistus*, in addition to the achromatic nucleolus, the advocates of the sex determination hypothesis could justly claim it as convincing evidence in support of their theory of its female origin, even if a demonstration of its relation to one of the chromosomes was lacking.

The absence of a chromatic nucleolus in these germinal vesicles cannot be due to the technique since in this case the more delicate achromatic nucleolus would show some disturbance. This point is exemplified in the germinal vesicle of *Allolobophora* (Photo 31), for in this preparation the pricking has disturbed the large, less chromatic nucleolus, while the small, denser, chromatic nucleolus remains perfectly intact, and this condition holds true for the hundreds of germinal vesicles of *Allolobophora* we have preserved.

It therefore seems fair to assume that the technique is not responsible for the absence of a chromatin nucleolus in the germinal vesicles of *Euschistus*.

In an earlier paper [1] we ventured to call attention to the likeness of the chromatin nucleolus in the egg of *Allolobophora* to the chromatin nucleolus of the first spermatocytes of insects. Compare the chromatin nucleolus in *Euschistus*, Photos 1 to 10, with the chromatin nucleolus in the egg of *Allolobophora*, Photos 31 and 32. In both these forms the chromatin nucleolus stains as

[1] Foot and Strobell ('05).

intensely as the chromosomes, and in both forms we sometimes find one and sometimes two chromatin nucleoli.[1] The comparison may be carried even a step further. We may compare the chromatin nucleolus of *Allolobophora* to the chromatin nucleolus of some forms where it is figured at one pole of the first spindle as an undivided chromosome. The chromatin nucleolus of *Allolobophora* often persists through the first division, and in such cases it is either free in the cytoplasm, near the spindle, or at one pole of the spindle in which position it strongly resembles the undivided chromosome figured in so many forms. Photo 32 shows a section through the first maturation spindle of an egg of *Allolobophora*, and a comparison of the chromatin nucleolus at the upper pole of this spindle, with the chromatin nucleolus in the germinal vesicle of Photo 31 leaves no doubt of the identity of the two structures. The identification is further confirmed by the clear demonstration in this and the adjoining sections of all eleven chromosomes in the equatorial plate of the spindle. If the chromosomes of *Allolobophora* were spherical as they appear in sections of so many forms, we could not so easily identify the chromatin nucleolus, for it stains exactly like the chromosomes. Such a case is described by Arnold ('08) in the spermatogenesis of the Coleopteran *Hydrophilus piceus*. During the rest stage the chromatin nucleolus of this form is easily recognized, but he finds it impossible to differentiate it from the chromosomes during the first prophase, because the chromosomes at this stage are also spherical and both structures stain alike. He is able to identify it again at the first metaphase, where it lies in the cytoplasm near the spindle, or within the spindle at one pole. It persists through the first division, but disappears before the second mitosis.[2] It is clear that in its origin, behavior and time of disappearance the chromatin nucleolus of the spermatocytes of *Hydrophilus* closely resembles the chromatin nucleolus of *Allolobophora*, with the exception that the latter does not invariably persist through the first division. Arnold ('08) supports Moore and Robinson's ('05) conclusion that the chromatin nucleolus of

[1] Foot and Strobell ('05), Photo 115.

[2] Wheeler ('97) figured the nucleolus of the germinal vesicle of *Mysostoma glabrum* persisting through the second cleavage and he says that in some cases it persists through the next stage, and perhaps even later, page 35.

the first spermatocyte is not a chromosome and does not take part in either division. His evidence for this is very convincing, he says, if it is to be interpreted as an accessory chromosome which remains undivided in the first spindle it should divide in the second spindle, and half the second spindles should show one more chromosome than the other half. Arnold finds, on the contrary that the number of chromosomes in the second spindle is as constant as the number in the first. He gives in the following table the results he obtained:

Out of 100 counts of first meiotic:

85 per cent. gave..... 15 gemini and the nucleolus.
7 " " " over 15 " " " "
8 " " " under 15 " " " "

Out of another 100 counts of first meiotic:

90 per cent. gave..... 15 gemini and the nucleolus.
3 " " " over 15 " " " "
7 " " " under 15 " " " "

Out of 100 counts of second meiotic:

91 per cent. gave..... 15 chromosomes.
6 " " " over 15 "
3 " " " under 15 "

The resemblance between the chromatin nucleolus of the first spermatocyte of insects and the chromatin nucleolus in the germinal vesicles of some forms has been noted by Häcker ('07) who says, that in those cases in which the heterochromosome is figured as a cap-shaped mass on a large pale plasmosome, the two structures are extraordinarily like the two kinds of nucleoli in the germinal vesicles of the lamellibranch type. He says: "So kann man sich dem Eindruck nicht entziehen, das z. B. das, 'akzessorische Chromosom' in den ruhenden Spermatocyten von Scolopendra (Blackman, 1905, tab. 2, fig. 10–15) früher einfach als 'Nucleolus' beschrieben worden wäre, und man ist geneigt, sich zu fragen, ob denn in allen vorliegenden Angaben bereits eine reinliche Scheidung zwischen den Heterochromosomen und den echten Nucleolen (Plasmosomen) durchgeführt ist. Es sei mir auch gestattet, darauf hinzuweisen, das die von Wilson u. a. gegebenen Bilder, in welchen ein Heterochromosom als

kappenförmige Masse einem grossen Plasmosom aufgelagert erscheint, eine auserordentliche Ähnlichkeit mit den Befunden in den Keimbläschen des Lamellibranchiaten-Typus zeigen, d. h. in denjenigen Keimblässen, in welchen sich zweierlei Nucleolen von verschiedener Lichtbrechung, Quellbarkeit und Tingierbarkeit befinden."

It is a suggestive fact that in *Allolobophora* we have an hermaphrodite form, and if one is determined to regard the chromatin nucleolus of the spermatocytes as a sex-determinant, some interesting conclusions might be drawn from a comparison of the two types of nucleoli observed in this hermaphrodite form, with the two types characteristic of the male and female cells in *Euschistus*. Compare for example the large nucleolus in the germinal vesicle of *Allolobophora* (Photo 31) with the large achromatic nucleolus of the germinal vesicles of *Euschistus* (Photos 18 to 30), and compare further the smaller chromatin nucleolus of *Allolobophora* (Photos 31 and 32) with the chromatin nucleolus of the spermatocytes of *Euschistus* (Photos 1 to 10).

Even if we could be sure that in *Euschistus* the achromatic nucleolus of the egg cells is not represented in the male cells, and that a striking likeness exists between the male and female nucleoli of *Euschistus* and the two types of nucleoli in the germinal vesicles of *Allolobophora*, we would still be unable to claim from the comparison any results of general significance, for the reason that the observations of many investigators of the spermatogenesis of insects offer contradictions to an assumption that the achromatic nucleolus is associated solely with the female cell — these observers having figured a pale, achromatic nucleolus in the spermatocytes, in addition to the chromatic nucleus characteristic of these cells. As opposed to these facts, however, other investigators have found no structure in the spermatocytes that can be interpreted as a nucleolus, other than the characteristic chromatin nucleolus.[1] These conflicting observations, if equally reliable, compel the conclusion that individual forms may differ as to the absence or presence of an achromatic nucleolus.

[1] In his "Studies on Chromosomes — IV.," *Jour. Exp. Zoöl.*, Vol. VI., No. 1, 1909, Wilson in describing *Syromastes*, does not mention a large pale plasmosome, such as he has described in other forms. In *Pyrrochoris* he states that there are from one to three nucleolar-like bodies, which on account of the staining reactions, he believes to be plasmosomes, page 82.

In *Euschistus* we have not been able to demonstrate its presence at any stage of the growth-period of the spermatocytes. In sections we often find faintly staining areas that might be interpreted as an achromatic nucleolus, but in view of the possibility of artefacts in such preparations, we hesitate to interpret them as true nucleoli, unless we can support the interpretation in our smear preparations. Until this point can be settled we are not justified in drawing any conclusions from the obvious difference in type between the nucleoli in the male and female cells of *Euschistus*, though we have here quite as marked a sexual difference of the nucleoli, as any that has been shown for the chromosomes, a difference that appears in no way associated with the maturation divisions of either germ cell.

In comparing the achromatic nucleolus of the oöcytes of *Euschistus* with the principal nucleolus of *Allolobophora* we find some individual differences between the two. The principal nucleolus of *Allolobophora* is clearly differentiated in the young oöcytes as a small, dense chromatic body, and it can be traced uninterruptedly through the entire growth period of the oöcytes. During this period it increases in size, proportionately to the growth of the nucleus, gradually becomes less dense and less chromatic, and finally, when the germinal vesicle has reached its maximum size the principal nucleolus often appears as in Photo 31. In some cases, however, its dense and chromatic character, distinctive of the earlier stages, persists until the chromosomes are fully formed or again it may so completely disintegrate that only a clear space remains as evidence of its existence.[1]

The achromatic nucleolus of *Euschistus* differs from the principal nucleolus of *Allolobophora* in not being present in the young oöcytes as a small dense chromatic nucleolus. We have been unable to demonstrate any such chromatic body in the young oöcytes of *Euschistus*. In the youngest stages in which a nucleolus is found, it appears as clearly achromatic as when it has reached its maximum growth in the germinal vesicle — compare Photos 13 and 16 with Photos 18 to 30. There are also indications that it is often not formed until after the oöcytes have attained a definite growth, for in such clear preparations as are shown in

[1] Foot and Strobell ('05), Photo 122.

Photos 11, 12, 14, 15 and 17, there is no evidence that such a structure is present.[1]

If the nucleolus in the germinal vesicle of *Euschistus* corresponds to the principal nucleolus of *Allolobophora* it must be conceded that there are marked individual differences between the two, differences quite as marked as those existing between the chromosomes of these two forms.

Individuality and Continuity of the Chromosomes.

The investigators who question the individuality and continuity of the chromosomes urge the necessity for further study of the critical stages bearing on this problem — the stages occurring between the end of one mitosis and the beginning of the next. Meves ('08) holds that during these stages it is impossible to recognize the chromosomes. He says: "Man sucht hier, wie O. Hertwig 1890 geschrieben hat, in den Kern etwas hineinzudemonstrieren, was kein unbefangener Beobachter in seiner Struktur erkennen wird.

Fick ('07) claims, that a study of the rest stages proves that the theory of the individuality and continuity of the chromosomes is untenable.

Tellyesniczky ('07) also makes a strong plea for more thorough and careful work on these stages. We are in sympathy with him in his skepticism concerning the individuality and continuity of the chromosomes, but our results differ from his in some, perhaps unimportant, details — differences which may normally exist in different forms. For example Tellyesniczky ('05) believes that a homogeneous distribution of the nuclear substance throughout the nuclear vesicle precedes every mitosis, and the young spermatocytes of our Photos 1, 4 and 5 lend support to this claim. The young oöcyte of Photo 13 also indicates a diffused condition of the chromatin, but as a rule the chromatin of the young germinal vesicles appears as fine granules often very evenly distributed throughout the nucleus as in Photos 11, 14 and 17. Possibly this granular condition may be an artefact — one phase of a precipitate left after drying, for in some cases the

[1] Among recent observations on this point, Deton ('08) finds in *Thysanozoön Brochii* that the nucleolus is absent in the young oöcyte, first appearing at the beginning of the growth period.

chromatin of the young oöcytes is as homogeneous as that of the spermatocytes of Photos 1, 4 and 5.

We find nothing in these germinal vesicles answering to Tellyesniczky's karysomes, unless he would homologize the aggregations of chromatin shown in Photos 12, 15 and 16 with these structures. These aggregations of chromatin are too numerous to be interpreted as the seven bivalent chromosomes of the later stages, or as their fourteen component univalents. In the germinal vesicle of Photo 15, for example, there are about fifty clumps of chromatic substance. Perhaps these clumps of chromatin correspond more closely to the segregated granules Wassilieff ('07) describes in the nucleus of *Blatta*. These clumps, he says, are too numerous to be interpreted as representing individual chromosomes. Later he finds that they disintegrate and are scattered throughout the nucleus like a fine dust; the delicate threads of the later stages being formed at the expense of this dust-like substance.

In the germinal vesicles of *Euschistus variolarius* the chromosomes first appear as extremely fine threads, which seem to arise as a concentration of the distributed granular mass, these granules gradually disappearing as the threads become more defined. Photo 17 shows a germinal vesicle at the stage just before the chromosomes appear — the chromatin is still distributed as granules throughout the nucleus, although the size of this nucleus is nearly equal to many of the germinal vesicles in which the chromatic threads are fully developed. In Photos 18 and 20 we see the delicate chromatic threads just appearing, as yet very indistinct, and in the later stage shown in Photo 21 the threads are more distinct but too much tangled to warrant any precise interpretation. In later stages, however, we can often trace among the filaments, an unbroken thread, so long that it cannot possibly be interpreted as one of the fourteen univalents, it must represent at least two univalents attached end to end. Again some of these threads are long enough to justify the inference that they represent even more than a bivalent, indicating that in *Euschistus* the chromosomes emerge from the rest stage not as univalents, but as long threads representing at least one or more bivalents, thus supporting the observations of those who believe that bival-

ents arise by the omission of a transverse division of a spireme, rather than by a conjugation of univalents. We believe that the evidence in *Euschistus* is opposed to the interpretation that the chromosomes first appear as univalents and later conjugate, either longitudinally or end to end. We believe, rather, that there is a tendency to form a continuous spireme, because we find cases in which very long, thin, unbroken threads are present, and a few examples of this kind outweigh as evidence, a large number of cases where we find the threads broken into small pieces. These delicate threads could easily be broken by the technique, for after pricking, the germinal vesicles flatten and dry on the slide, and this disturbance, combined with the shrinkage of drying, could easily account for the rarity of the cases in which the long thin threads remain unbroken.

In Photo 24 we see this tendency to the formation of a spireme — a few of the threads can be traced certainly too far to justify any assumption that they are univalent or even bivalent chromosomes. A long unbroken thread is also demonstrated in Photos 23, 28 and 29.

Tellyesniczky ('07) claims that the long thin chromatic threads do not become shorter and thicker by contraction. He thinks rather this is accomplished by a flowing together and change of place of the substance of which the thread is composed. He says, there is a continual disintegration and rebuilding of the threads and the new structure is formed at the expense of the old.

We believe this is not the case in *Euschistus*. For example, it seems reasonable to interpret the chromosomes of Photo 30 as due to contraction of long thin bivalents (as shown in Photo 25), rather than to regard them as entirely new formations. Such a detail as this is interesting, but it seems to us only incidental to the all-important question, do the chromosomes retain their individuality throughout the rest stages — do they differ in this particular from other structures of the cell — the centrosome — the aster — the spindle — the nucleolus, etc. These elements return at each cell generation with as much regularity as to size and form as do the chromosomes, but those who believe in the individuality and continuity of the chromosomes attribute to the latter structures a causal significance not now ascribed to any other organ of the cell.

In the past, however, similar claims have been made for the centrosome — its individuality and continuity were asserted by many cytologists, some going so far as to believe the centrosome to be the cause rather than the expression of cell activity.

Certain facts indicate that all the chromatic substance of the germinal vesicle of *Euschistus* is not used for the chromosomes. We find in many germinal vesicles various segregations of chromatic substances which are present after the chromosome threads are formed and which often do not entirely disappear until the prophase stage. We sometimes find irregular granular or homogeneous masses as in Photos 20 and 22, sometimes numerous large granules, so dense that they look like very small nucleoli (Photos 19, 21, 23, 26, 27 and 29), and sometimes merely deeply staining areas, as shown in Photos 23, 24 and 27. We have no means of proving that this chromatic substance, not used for the chromosomes, is chromatin, but for many forms it has been claimed that only a small part of the chromatin is used in the formation of the chromosomes and we are therefore perhaps justified in surmising that the chromatic substance in *Euschistus* (which often persists until after the chromosomes are formed) may be chromatin residue, which assumes various forms during its disintegration and disappearance.

The early stages of the development of the germinal vesicles shown in Photos 11 to 17 we interpret as indicating that the chromosomes completely disintegrate — that any claim of their morphological persistence during these stages must be made as a pure assumption, as there is no morphological evidence whatever of their persistence. On the contrary there is every indication that they completely disintegrate. We believe we have no reason to assume that the reappearance of the chromosomes at certain stages differs essentially from the reappearance of the centrosome, aster, spindle, nucleolus and other cell structures.

Fick ('07) in his critical review of the inconclusive evidence that has been offered as proof of the individuality and continuity of the chromosomes, and of their causal character, sounds a welcome note of protest against recent chromosome speculations. In addition to Fick such experienced cytologists as Häcker and Meves, have expressed their skepticism of the sex-

determination hypothesis in no uncertain terms, and many of the investigators who have studied the accessory chromosomes in insects are equally incredulous, Montgomery, Schäfer, Moore, Robinson, Arnold and others.

We have reserved a description of the later stage of the germinal vesicle chromosomes of *Euschistus* for a subsequent publication, which will discuss only the chromosome groups in both male and female cells. In the male cells we have traced the idiochromosomes through both maturation divisions and are able to support, in the main, Wilson's results as to their division in these stages. He says, the idiochromosomes remain univalent in the first division, each dividing independently, and in the second division the two separate — the small idiochromosome going to one pole and the large idiochromosome to the opposite pole. He gives no details however as to the method of the first division and as we find an interesting irregularity in this division, we believe it is worthy of consideration. As the idiochromosomes separate in the second spindle, this is presumably the so-called reduction division for this unequal tetrad, and the first division should be therefore an equation division, and *both* idiochromosomes should divide longitudinally, if the significance that has been attributed to the longitudinal and transverse divisions of the chromosomes holds true in this form.

The value of the significance of a longitudinal or transverse division is based on the assumption that the so-called ids are arranged in a row, and a morphological demonstration of this assumption is claimed for those cases in which the chromosomes are rod- or thread-shaped — these rods or threads often appearing as a single row of chromomeres.

In our smear preparations of the testes of *Euschistus variolarius* both the large and small idiochromosomes are distinctly rod-shaped, and at the late first prophase and metaphase it can be clearly demonstrated that the *large rod divides longitudinally and the small rod transversely*. There are rare (perhaps only apparent) exceptions to this, but as a rule one divides transversely *just as surely and as constantly* as the other divides longitudinally, and this we have demonstrated in a number of photographs.

The germ cells are being studied in order to obtain a morpho-

logical basis for conclusions or to put conclusions to a morphological test, and the idiochromosomes seem to offer a fruitful test of the theoretical value of longitudinal and transverse divisions. In order to retain our faith in the special significance that has been attributed to the longitudinal and transverse divisions we are driven to the inconsistency of accepting the evidence in the case of one idiochromosome, and disclaiming it for the other — though the evidence in both cases is equally strong. If, on the other hand, we accept the evidence and at the same time retain faith in the theory, we are forced to admit that the sister cells of the first division are also dimorphic and we have a so-called reduction division of one of the idiochromosomes of the first spindle as surely as we have a reduction division (the separation of the two idiochromosomes) in the second spindle, we thus have a somewhat similar phenomenon occurring in both spindles.

We have a number of photographs demonstrating both spermatocyte divisions and also a large number showing the spermatogonial, oögonial and embryonic chromosome groups — several hundred in all, but the publication of these must be reserved for a separate paper, where we shall aim to make a very full comparison of these chromosomes, giving especial attention to the groups that have not been presented in the papers which advocate the sex-determination hypothesis. We believe that these groups are of special value — that we have no right to ignore exceptions because they do not fall in line with certain theories. The trite claim that exceptions prove the rule loses its force when enough exceptions are found to challenge the rule. A careful examintion of our preparations makes it possible to select chromosome groups which exactly fit a given theory, but many groups can also be found that are a serious menace to these theories, while on the other hand they present no difficulties to the conception of those who regard the number, size and form of the chromosomes as inherited characters — the expression of cell activities rather than the cause.

January, 1909.

Appendix.

We have just received Wilson's latest "Study on Chromosomes," No. V.,[1] in which (in a footnote on page 159) he attempts to explain what he calls our "entirely mistaken conclusions" regarding the division of the accessory chromosome in *Anasa tristis* as follows:

"The regrouping of the chromosomes in the second division, first described by Paulmier ('99) in *Anasa tristis*, is characteristic of the Coreidæ generally, an eccentric position of the idiochromosome being a nearly constant feature of the first division but not of the second. Failure to recognize this fact in the case of *Anasa tristis* seems to have been one of the main sources of error in the entirely mistaken conclusions of Foot and Strobell ('07*a*, '07*b*) regarding this species. (Cf. Lefevre and McGill, '08.) Demonstrative evidence on this point is given by polar views of rather late anaphases, in which every chromosome of each daughter plate may be seen, in the same section. Such views, of which I have studied many, both in *Anasa* and other genera, show that one of the chromosomes may indeed occupy an eccentric position, and may there divide; but in such cases the odd chromosome is always found elsewhere in the group, lying either in or near one of the daughter groups and not in the other. When the odd chromosome is eccentric it is found in one of the daughter groups but not in the other."

In order to speak with authority on the regrouping of the chromosomes, some accuracy at least is expected in the identification of the individual chromosomes. Paulmier's authority can be dismissed when we recall Wilson's interesting discovery that in *Anasa tristis* Paulmier erroneously identified the microchromosome as the accessory (undivided) chromosome of the second spindle.

In Plate III. of our "Study of Chromosomes in the Spermatogenesis of *Anasa tristis*" ('07) we *show* in Photos 27, 28, 33, 35, 36, 37, 38, 39, 42, 43, 44 and 45, late anaphases or early telophases of second spindles demonstrating the ring-like formation of the chromosomes, with one of the chromosomes eccentrically placed or lagging. In our smear preparations this grouping is

characteristic of the second division as it is of the first, although, as we have already pointed out, there are exceptions to this rule. There can be no possible doubt of our identification of these groups as second spindles, because they are invariably found in regions of the testis surrounded by spermatids in different stages of development and, further, the form of the chromosomes is so beautifully demonstrated in our smear preparations, it is hardly possible to confound the first and second spindles,[1] a mistake easily made in sections. That the individual chromosomes may change their relative positions at this stage, while the form of the group remains unchanged, is a possibility that any observer would naturally consider. But as we find in our preparations, the ring-like grouping of the chromosomes of the first spindle so constantly duplicated in the second spindle, with the micro-chromosome (the one chromosome that can be identified beyond question) maintaining its characteristic position in the center of the ring, the identification of the eccentric chromosome of both spindles as *probably* the same chromosome, is certainly as legitimate a conclusion, as forcing an arbitrary contradiction of this assumption, in order to support a theory.

In complete opposition to what Wilson calls his "demonstrative evidence" on this point, we refer to our published photographs, Plate III. (in the paper quoted above), 29, 32, 33, 34, 35, 36, 37, 40, 41, 43, showing polar views of late anaphases or telophases of second spindles, in which every chromosome of each daughter plate can be counted. In all these preparations there is an eccentric, lagging — in most cases dyad — chromosome, but as *ten* chromosomes can be clearly counted at each pole, we fail to see how the situation is improved, for Wilson to claim, that where the lagging chromosome divides in this spindle it is not to be identified with the "odd" chromosome — which he says, "is, in such cases, *always to be found elsewhere in the group.*" We have given demonstrative evidence that this is not so in our preparations.

The essential fact is not the relative position of the accessory chromosome in either the first or second spindles, but whether

[1] The ring-like grouping of the chromosomes is also characteristic of the first and second spindles of *Euschistus variolarius*, where the distribution of the unequal tetrad in the second spindle adds conclusive proof of the identity of the spindles.

one of the chromosomes consistently fails to divide in the second spindle. It has been said of the accessory chromosome that in the spindle in which it is heterotropic, it tries to divide, but does not succeed. In the case of *Anasa tristis*, we have demonstrated that it is by no means always unsuccessful in these efforts.

We regret that we cannot agree with Professor Wilson as to the value of the support he believes he has received from Lefevre and McGill's paper on *Anasa tristis* and *Anax junius* ('08). In this paper our results in *Anasa* are summarily disposed of in two pages of curt contradictions, supported by ten sketches made from sections. The futility of such methods as opposed to the very full demonstration we have given in 107 photographs to support our conclusions in *Anasa*, must be apparent to any unprejudiced observer.

In this same paper, McGill corrects her earlier observations on *Anax junius* on three very important points :

1. In her first work on this form ('04) (in which she expresses her indebtedness to Professor Lefevre) she found no evidence of a chromatin nucleolus in the resting spermatocyte. She and Lefevre now find, *in the same material*, a chromatin nucleolus persisting through the resting spermatocyte.

2. In her earlier work McGill identified the microchromosome as the accessory chromosome. She and Lefevre now identify, *in the same material*, one of the larger spermatogonial chromosomes as the accessory chromosome.

3. In her original count of the spermatogonial chromosomes, McGill found an even number, 28. She and Lefevre now find *in the same material* an odd number, 27 spermatogonial chromosomes.

In view of these contradictions we may justly hesitate to accept as definitive the recent conclusions reached by McGill and Lefevre in *Anasa tristis*, believing a new point of view may give us still further variations in their very interesting observations.

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Explanation of Plates.

All the photographs were taken with a Zeiss Apo. 2 mm. immers. lens, 140 apr. and compensating ocular 4. Photos 1 to 31 are from smear preparations stained with a saturate solution of Bismarck brown. In all these preparations the *whole nucleus* is shown.

Magnification of Photos 1 to 10 inclusive, 1,000 diameters.

Magnification of Photos 11 to 32 inclusive, 600 diameters.

As some of the germinal vesicles were too large to be included in the field at a magnification of 1,000 diameters, we used a magnification of 600 diameters for all the oöcytes, in order to facilitate a comparison.

The reproductions were made by the Rotograph Company from our own negatives.

Plate I.

Euschistus variolarius.

Photo 1. Two young resting spermatocytes, first order, one showing two nucleoli and the other one nucleolus.

Photo 2. A little later stage than Photo 1. Two nucleoli are shown, unequal in size, but this inequality is not so marked as in the two nucleoli of Photo 6.

Photo 3. Resting first spermatocyte about the same stage of development as Photo 2. One nucleolus present.

Photo 4. A resting first spermatocyte showing two nucleoli about equal in size.

Photo 5. A resting first spermatocyte with two nucleoli, unequal in size, though the inequality here is not so conspicuous as in Photo 6.

Photo 6. A later stage than Photo 5. The chromatin shows the segregation which precedes the formation of the chromosomes. Two nucleoli are present, very unequal in size.

Photo 7. First spermatocyte with the chromatin somewhat more closely segregated than in Photo 6. One nucleolus is present showing a typical vacuole.

Photo 8. A larger first spermatocyte with one nucleolus, the disposition of the chromatin suggesting a network.

Photo 9. A first spermatocyte showing an early stage in the formation of the chromosomes; one nucleolus is present.

Photo 10. A much later stage than Photo 9. The bivalent chromosomes are formed, but the number is not so clearly demonstrated as at later stages. (These later stages, as well as the transitional stages, between Photos 9 and 10, are reserved for a subsequent publication.) The nucleolar-like thickening in the loop of the chromosome on the lower periphery of the group is not a small nucleolus. In the preparation it can be plainly recognized as a segregation of chromatin at the point where a loop of the chromosome brings two parts of the thread in contact. Similar, though less conspicuous thickenings are seen at other points where two threads cross. One clear nucleolus is present in this spermatocyte.

Photo 11. Nucleus of a young oöcyte, with the chromatin granules quite evenly distributed throughout the nucleus. No nucleolus present.

Photo 12. A slightly older germinal vesicle, with the chromatin segregated into clumps, too numerous to represent individuual chromosomes.

Euschistus variolarius. PLATE I

FOOT & STROBELL PHOTOS.

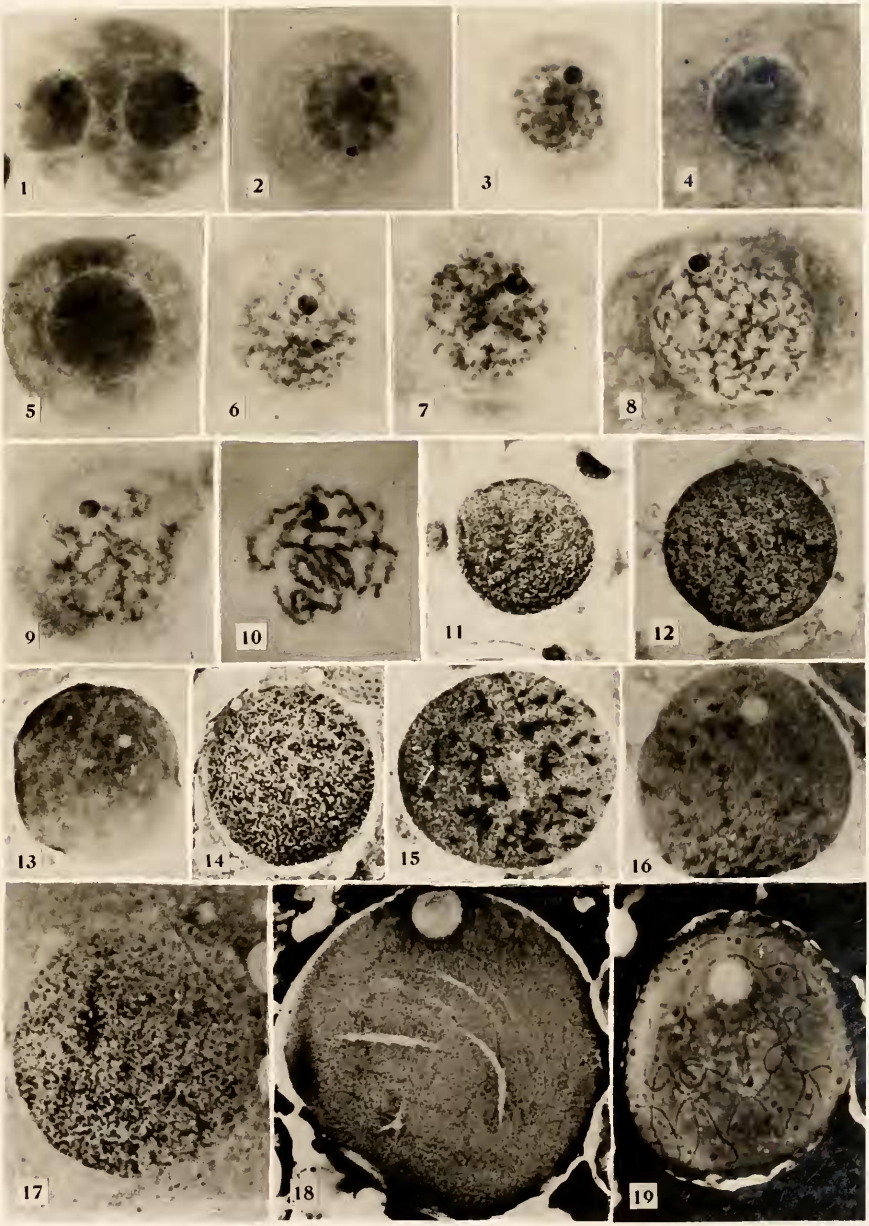


PHOTO 13. A young germinal vesicle with the chromatin almost in a diffused condition. A small, clear, spherical space is present, which may represent the first appearance of the achromatic nucleolus so conspicuous in the older germinal vesicles of Photos 18 to 30.

PHOTO 14. A young germinal vesicle with the chromatin evenly distributed as in Photo 11.

PHOTO 15. An older germinal vesicle with the chromatin segregated into numerous clumps as in Photo 12. Many of the clumps in this germinal vesicle appear almost as dense as nucleoli, though in the preparation they are seen to be merely a close segregation of fine granules.

PHOTO 16. An older germinal vesicle showing less pronounced segregations of chromatin, and a clear spherical space which can surely be interpreted as an early appearance of the achromatic nucleolus seen in the germinal vesicles of Photos 18 to 30.

PHOTO 17. A much larger germinal vesicle with the chromatin quite evenly distributed as granules.

PHOTO 18. An older germinal vesicle with the first indication of the formation of delicate chromatic threads, which later develop into the chromosomes. At this stage the achromatic nucleolus is always present. The cracked spaces shown in this germinal vesicle and in Photos 21, 23, 24 and 29 are due to uneven shrinkage as the germinal vesicles rapidly dry on the slide.

PHOTO 19. A much later stage than Photo 18 — the chromatin threads being well formed. (The next stage to Photo 18 is shown in Photo 20, Plate II.) The germinal vesicle of this preparation is not well flattened and some of the chromatic threads are very much out of focus.

Plate II.

Euschistus variolarius.

Photo 20. A germinal vesicle a little further developed than the one shown in Photo 18. The delicate chromatic threads are a little more defined. The achromatic nucleolus is present near the periphery.

Photo 21. A germinal vesicle with the delicate chromatic threads well formed. The achromatic nucleolus is seen on the periphery.

Photo 22. A germinal vesicle with the chromosome threads further developed. The nucleolus near periphery.

Photo 23. A germinal vesicle with long, thin chromosome threads. The nucleolus on periphery.

Photo 24. A germinal vesicle at about the same stage of development as Photo 23 The nucleolus on periphery.

Photo 25. A germinal vesicle with the chromosome threads further developed.

Euschistus variolarius.

FOOT & STROBELL PHOTOS.

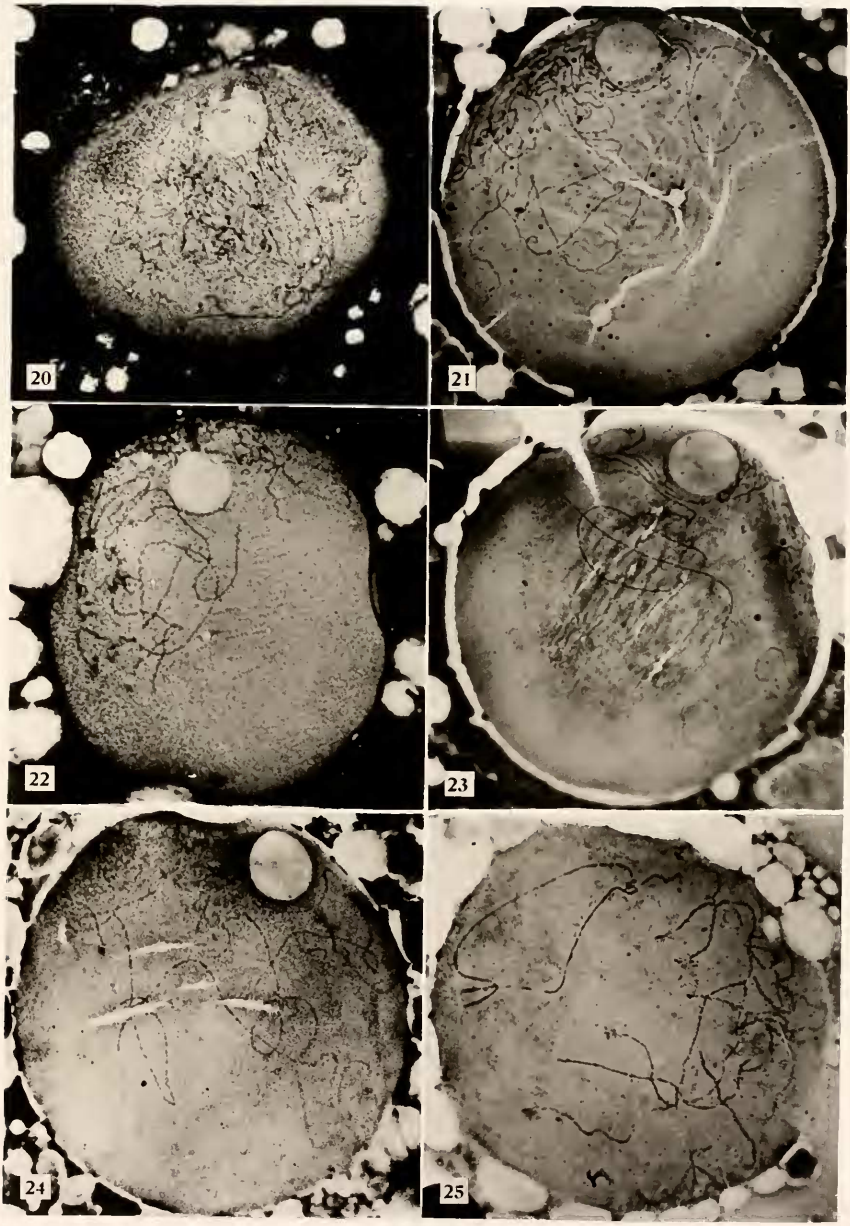


PLATE III.

Euschistus variolarius and Allolobophora fœtida.

PHOTO 26. A germinal vesicle with chromosome threads well formed. Nucleolus on periphery.

PHOTO 27. A germinal vesicle at about the same stage of development as Photo 26. The nucleolus near the center.

PHOTO 28. A germinal vesicle with long, thin, twisted chromosome threads. Nucleolus near the center.

PHOTO 29. A germinal vesicle showing a later stage of development. The chromosome threads have contracted into shorter, thicker pieces. Nucleolus still present.

PHOTO 30. A germinal vesicle with the chromosomes almost at first prophase stage. Nucleolus still persisting.

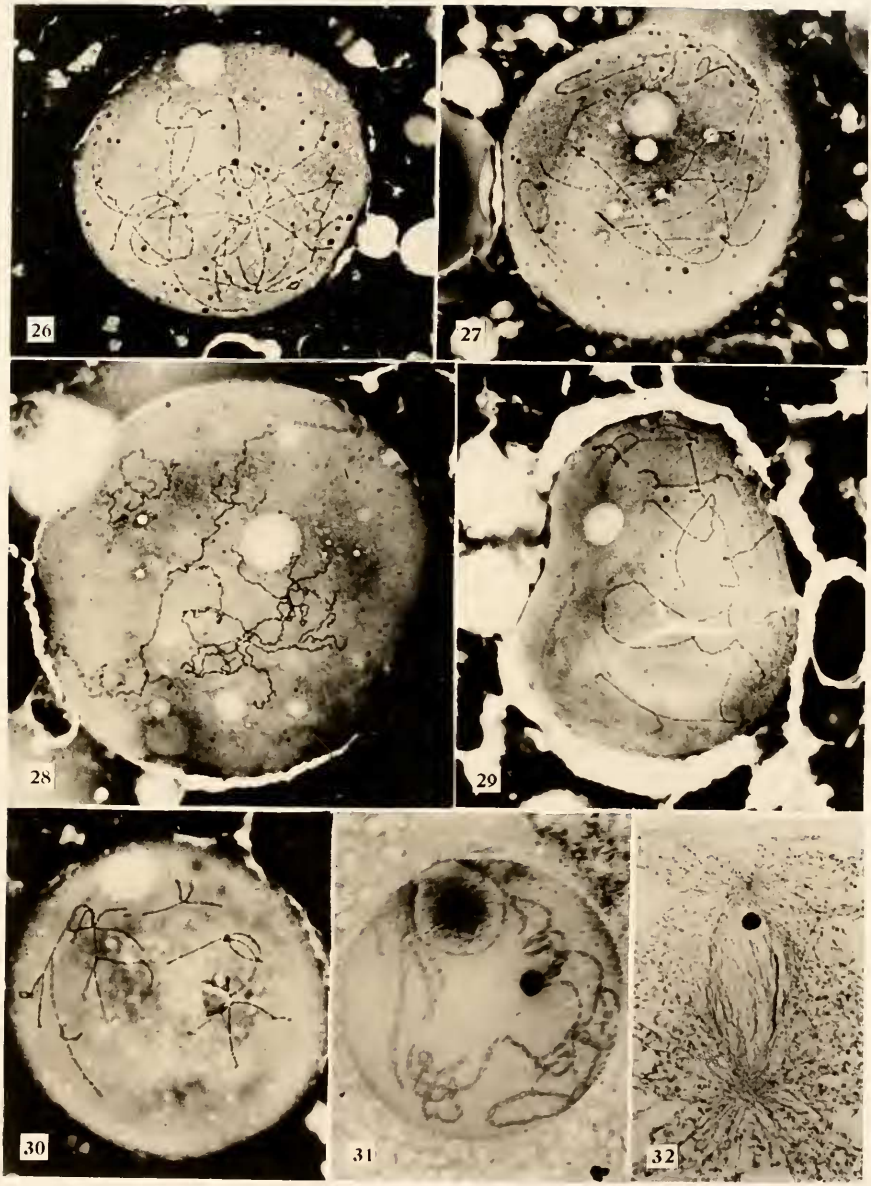
PHOTO 31. A germinal vesicle of *Allolobophora fœtida*. The same technique as that used for the *Euschistus* germinal vesicles (Photos 18 to 30). The chromosome threads are long, and thin, and longitudinally split. The large, less chromatic nucleolus is on the periphery, and the dense chromatin nucleolus is near the center.

PHOTO 32. A section (2½ μ thick) through the first maturation spindle of *Allolobophora fœtida*. At the equator one chromosome is shown, the ten remaining chromosomes being clearly demonstrated in the adjoining sections. Near the upper pole we see the persisting chromatin nucleolus. A centrosome is shown at each pole

Euschistus variolarius.

FOOT & STROBELL PHOTOS

PLATE III



INHERITANCE IN THE "WALKING-STICK," APLOPUS MAYERI.

CHARLES R. STOCKARD.

The family Phasmatidæ, as is well known, shows some of the most striking cases of "protective resemblance" found among the insects. Several of the genera are typically stick-like to a surprising degree while members of the genus *Phyllium* resemble in detail a leaf-like structure. These animals are no doubt protected by their imitative forms provided they behave in a certain manner. In fact the protection or concealment of such an animal depends as largely on its behavior as upon its resemblance to surrounding objects. In order to ascertain whether these so-called protectively adapted insects really exhibited a "protective behavior" I [1] studied the habits of the "walking-stick," *Aplopus mayeri*, which is abundant on the Tortugas Islands, Florida. These large insects were found to behave in a manner almost ideal for their concealment among the twigs and stems of the plant on which they feed, *Suriana maritima*.

My study was made during a season, June and July, when the enemies of *Aplopus* were extremely rare on these islands. In the spring and fall, however, the great numbers of migrating birds which stop here no doubt devour many of these large Orthoptera in spite of their almost perfect concealment. But for their protective resemblance and habits birds might easily exterminate such slow-moving flightless insects within a few seasons, in fact the existence of creatures like *Aplopi* on these small islands is really dependent upon their ability to be passed unobserved by birds migrating between the eastern United States, West Indies and South America.

The question arises whether the protective behavior in *Aplopus* is fully developed on hatching from the egg or whether it is attained with their large size and mature condition. In order to

[1] "Habits, Reactions and Mating Instincts of the 'Walking-Stick,' *Aplopus mayeri*," *Science*, N. S., XXVII., 1908 — Publication No. 103, Carnegie Institution, Washington, 1908.

investigate this and the further question of first-leg form considered below eggs were collected during the summer and brought to New York where the newly hatched individuals might be observed.

These eggs were kept in small loosely stoppered bottles in the laboratory at ordinary room temperature. They began hatching about the first of December and during January a large number of the insects came out.

Behavior of Newly Hatched Aplopi.

The reactions of the small insect on the day it emerges from the egg are almost identical with those of the fully mature eight-inch females which I studied at the Tortugas. The young *Aplopi* are a light chocolate-brown in color with yellowish bands about the legs and the sexes are similar. The adult males, however, become greenish in color while the female retains her original brown. The adults also have rudimentary wings which are capable of being raised when the insect is excited, but the young are wingless. Their reactions will be referred to briefly at this time as they are given in some detail in my former paper.

The insects when at rest among the twigs assume an attitude which in consequence of their stick-like shape makes them most difficult to detect. The first pair of legs are stretched directly forward enclosing the head between thin curved portions of the femora which fit perfectly against it. The antennæ are brought

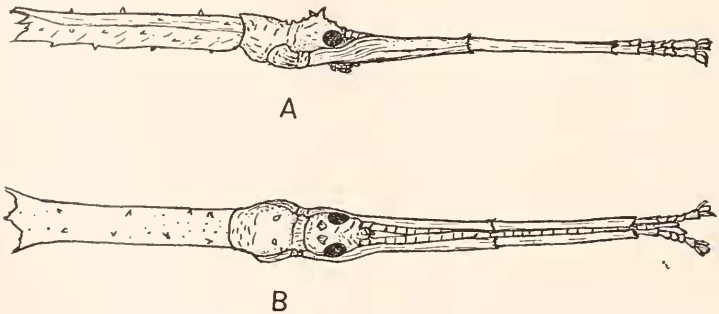


Fig. 1. *A.* Lateral aspect of *Aplopus*, head and first two thoracic segments. The first pair of legs are stretched forward in the typical resting attitude. The femur fits perfectly about the ventro-lateral region of the head and leaves the eyes uncovered.

B. Dorsal view of the same specimen showing the approximated antennæ directed forward and enclosed between the first pair of legs.

together between the first legs, Fig. 1, *B*. (See also Fig. 1, Plate I., and the photographs in my former paper.) The anterior end of the insect thus closely resembles a more or less pointed stick. The newly hatched individual habitually assumes this position and the point of especial interest, which I shall return to later, is that the thinned out curved part of the femora fit about the head as perfectly the first time the legs are stretched forward as they do in the adult.

In walking from place to place the adult moves slowly and often exhibits a slow laterally swinging motion suggesting a twig swinging in a light breeze. The young also swings its body from side to side in a similar manner. When a number of young *Aplopi* are sitting motionless if the observer blows a current of air over them they all begin to swing very actively from side to side as if being swung by the breeze. This swinging motion no doubt serves to render them less conspicuous among the shrubs.

The newly hatched individuals use the same methods to escape an enemy as those employed by the adult. When they are touched or pinched slightly they move away a short distance and immediately come to rest again, if the stimulus be repeated they begin to walk at a more rapid gait than before and move a greater distance away. If again touched they drop bodily to the floor and feign death just as the adult does. The death-feigning reaction is more readily induced in the young than in the adult, and no doubt serves to great advantage in enabling them to escape an enemy which fails to seize them securely in the first attempt. The chances of escape for this stick-like creature when it drops through the dense foliage and branches of the *Suriana* bush is most favorable. When in the death-feint the legs may be bent in any position and the body twisted without the least move on the part of the animal. They may actually be piled one on another and will remain as motionless as dead insects.

The young walking-stick crawls upwards on any object that it may reach after emerging from the egg. As I previously recorded the female *Aplopus* sits in the *Suriana* bushes and lays its eggs which fall to the ground where they later hatch. Thus the tendency of the young to crawl upwards on the first object with which it comes in contact serves to bring it up the *Suriana* bush

to its leafy food. In crawling up the young insect waves its antennæ to feel the way just as does the adult and reaches out with the first legs to grasp the object located by the antennæ.

Finally the young like the adult is more or less nocturnal in its movements. During the day they sit motionless with the first legs extended forward but at night they become active and move about to feed. The food of the adult is limited to the leaves of *Suriana*. I have made no attempt to feed the young since they may be kept alive for about one week after hatching without taking food.

The Thin Curve of the Femora which Fits Against the Ventro-Lateral Surfaces of the Head.

When the first pair of legs are extended forward the femur of each is so curved near its proximal joint as to fit perfectly against the ventro-lateral parts of the head and at the same time leaves the eyes uncovered, Fig. 1, *A* and *B*. The curved portion of the femur is also very thin in a lateral direction and thus when pressed closely to the head the legs go out as almost straight lines instead of bulging around the head to any great extent. It seems difficult to believe that the first pair of legs could through chance variations or mutations have come to fit so perfectly around the sides of the head and at the same time to have their dorsal line so curved as to leave the eyes uncovered. It must be remembered that when the first legs are in the extended position the head presses against the dorso-lateral surfaces of the femur and not straight against the inner lateral surface only. This arrangement may be better understood by a close examination of the dorsal and lateral views given in Fig. 1, *A* and *B*.

The possibility suggests itself that the perfection of the fit is attained during the life of the individual since the legs are so habitually pressed against the head for about twelve hours daily. To test this it became desirable to study newly hatched individuals in order to find whether the femur curve was as perfectly adjusted in them as in the adult. A careful examination of about one hundred *Aplopi* shortly after emerging from the egg has convinced me that the curve of the femur is as true to the head pattern in the newly hatched young as in the mature insect when several months old.

Finally, is this adjustment between the structure of the femora and head due to the position of the insect when enclosed within the inelastic egg shell? If the first legs were folded forward against the head the pressure during embryonic life might easily be sufficient to mold the femur curve into pattern for the head. Twenty eggs containing embryos at various stages shortly preceding hatching were dissected with this question in view. The elliptical egg shell is of a rigid chitinous material with a circular operculum at one end and a hilum-like scar on one side to which the

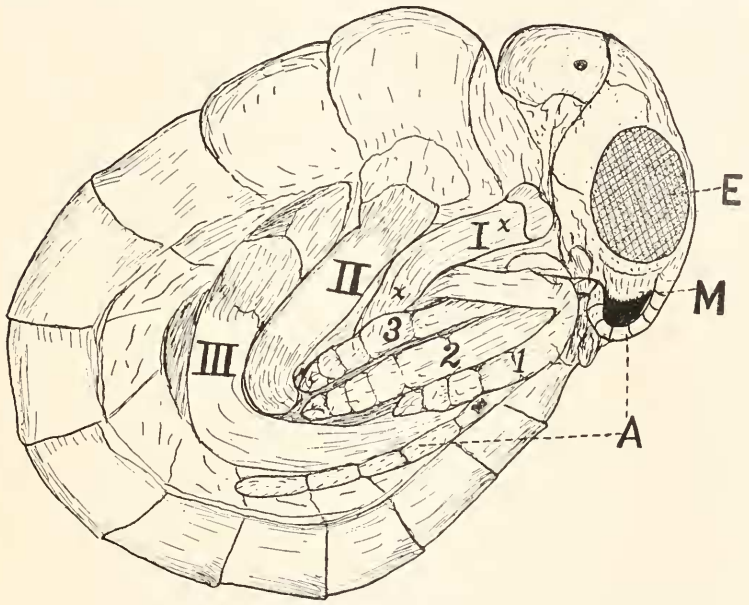


FIG. 2. An unhatched *Aplopus* with its egg membranes dissected away showing its folded position. The femora of the three right legs are marked with roman numerals and the tarsal ends of the same legs are indicated by figures. The part of the first femur which in later life fits against the head is shown between the points *x–x*, it is not molded against the head in the egg. *A*, antennæ; *E*, compound eye; *M*, mandible.

inner egg membrane is attached. The size of the egg ranges from 2 mm. in narrow diameter by 4 mm. in long diameter to 3 mm. by 4.5 mm. The length of the walking-stick on hatching is from 17 mm. to 23 mm. measured from the tip of abdomen to tip of the first pair of legs when extended forward, or from tip of head to tip of tail 9.5 mm. to 13 mm. The embryo within the egg is, therefore, necessarily much folded and bent.

On dissecting the egg the embryo is found to be curled around in a rather constant manner, the head being usually, though not always, near the opercular end. The long legs are folded back and forth upon themselves in a very definite fashion as shown by the camera drawing, Fig. 2. The antennæ (*A*) pass down the front of the head and then back along the ventro-lateral surface of the abdomen being sometimes bent around the first pair of legs. The point of most importance is that the femoral segments of the legs are all directed obliquely away from the head. The first pair of legs each of which is folded on itself four times does not touch the sides of the head at all. The head and large eyes are entirely uncovered and exposed. The femora of the first pair of legs not only fail to mold their curves against the head but the femora are so pressed against the thorax that the surfaces which will subsequently be concave (in Fig. 2 between *x* and *x*) are actually arched convexly. Thus it is seen that the mechanical arrangement of the embryo's parts within the egg is not responsible for the fit of the femur curve against the head. On the contrary the curve seems to develop in spite of these arrangements.

When hatching the embryo's head and body come forth from the egg first, the antennæ are then pulled out, the legs being the last parts liberated from the shell, Fig. 3. It often happens that the shell is carried around for some time dangling to the third pair of legs. In Fig. 3 the well developed curves of the femora are distinctly shown, *x* to *x*, and are being pulled in a direction away from the head, yet as soon as the legs are free from the shell the first pair may be straightened forward and their curved femora fit neatly against the sides of the head. We see, therefore, that the curve of the femora to fit the sides of the head is a character transmitted to all of the young and perfectly formed at the time of hatching. It might seem that the origin of this character was most probably due to the habit of the insects to press the first pair of legs against the head. Gradually this pressure developed a thin concave region of the femur of the first leg which molded itself more and more perfectly to the contour of the head. If this curve arose in any other way the second and third pairs of legs might have developed at least a trace of such a character though this is not absolutely necessary. It must be

remembered that the curves fit the ventro-lateral contour of the head to a remarkable degree.

When the first pair of legs are so stretched forward the insect's antennæ are brought together. The legs have an irregular groove extending along the approximated surfaces and when complete approximation takes place a rather imperfect tube is formed enclosing the antennæ. This is a case analogous to the above and it is difficult to imagine how chance variations could

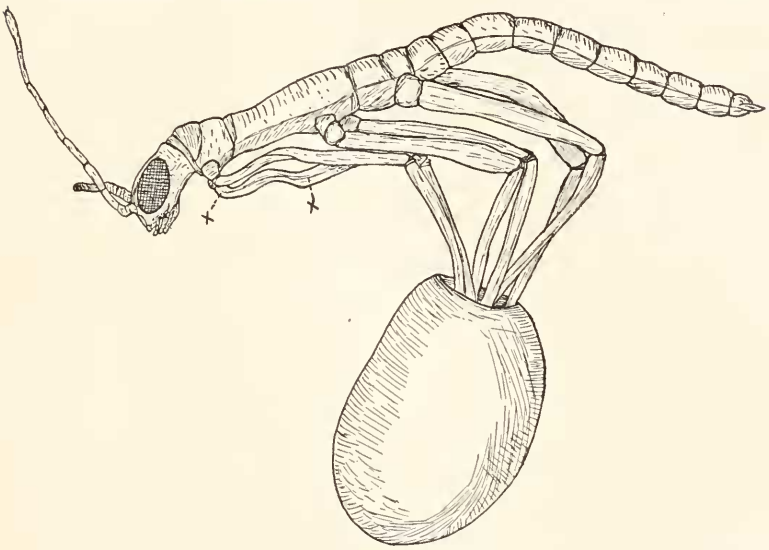


FIG. 3. *Aplopus* in the act of hatching from the egg. The body and head come out first, then the antennæ and finally the legs free themselves from the shell. The parts of the first legs between *x*–*x* are curved to fit the head when they are straightened forward although they have never touched the head up to this time.

bring about such mechanical harmonies between organs only associated through an habitual attitude assumed by the animal when at rest. Yet it must not be forgotten that many other equally as nice morphological arrangements exist which have no habit or action connected with them. Indeed a crucial case of use inheritance is almost impossible to imagine from purely descriptive work. I would not be understood as advocating any principle of inheritance but merely bring forward the present case as being of interest in itself.

CORNELL MEDICAL SCHOOL, NEW YORK CITY,
February 6, 1909.

THE CONNECTIONS OF THE GONADIAL BLOOD VESSELS AND THE FORM OF THE NEPHRIDIA IN THE ARENICOLIDÆ.

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The gonads of *Arenicola* occur on certain blood vessels that lie diagonally on the exterior of the glandular portion of the nephridia and that are consequently designated the gonadial vessels. In a study of the gonads, the results of which will be published shortly, it was found that the literature of the subject contained conflicting and inaccurate statements regarding the relations of these gonadial vessels to their connecting vessels. This paper is the result of an attempt to determine these relations.

Gamble and Ashworth, in 1900, published an extended study of the anatomy of the several species which also reviewed the important literature on the family. They had previously published, 1898, a paper on the anatomy of *Arenicola marina*. In an article published in 1899, V. Willem disagreed with some of their statements as did R. Lillie in a more recent publication, 1906. These contradictory statements I shall try to adjust: I must also take exception to some other statements of each.

The general relations of the blood vessels may be easily understood from the accompanying partly diagrammatic figure of a cross-section at about the level of the first nephridium of *A. cristata* (Fig. 1). It will be noted that there are two main blood vessels, a dorsal and a ventral; these run the entire length of the animal. There is a pair of neurals of much smaller caliber, also extending the full length of the body. The paired gastric laterals and subintestinals, as also the paired integumentary vessels known as the parietal (or dorsal longitudinal) and the longitudinal nephridial, are limited in their extent and vary in length and prominence in the several species.

All previous authors agree in the arrangement of the nephridial vessels as follows: The afferent vessel, on approaching the

nephridium, divides, one branch going to the setal sac and gill, if present, one to the integumentary vessels and one to the nephridium. The latter enters the nephrostome and forks, one branch traversing each lip. After reuniting, the vessel, reformed, runs out onto the wall of the nephridium as the gonadial vessel. Each of the three main branches of the afferent vessel gives off smaller vessels which, with more or less anastomosing with adjacent vessels, form capillary net-works in the organs supplied.

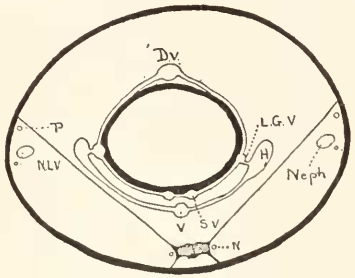

FIG. 1. Partly diagrammatic cross-section of *Arenicola cristata* at about the level of the first nephridium. *D.V.*, dorsal blood vessel. *H.*, heart. *L.G.V.*, lateral gastric blood vessel. *N.*, neural blood vessel. *N.L.V.*, nephridial longitudinal blood vessel. *P.*, parietal blood vessel. *S.V.*, sub-intestinal blood vessel. *V.*, ventral blood vessel.

The efferent vessels are formed by the union of capillaries in these same regions.

The afferent vessel, as a rule, comes from the ventral vessel. This is true for all except the first two nephridia of *A. Grubii* and of *A. ecaudata*, which nephridia are supplied, the first by a branch of the dorsal, the second by a branch of the parietal vessel (Fig. 2, *d*). In addition to the afferent vessel from the ventral vessel, the following nephridia also receive branches from the dorsal vessel: the first of *A. cristata*, the first two of *A. Claparedii* and the first three of *A. marina* (Fig. 2).

The following nephridia return blood directly to the subintestinal vessels through efferent vessels whose numerous branches are adjacent to their funnels: the fourth, fifth and sixth of *A. cristata*, the fourth and fifth of *A. Claparedii* and the fifth and sixth of *A. marina*. All others must pour the blood into the parietal and nephridial longitudinal vessels which, in turn, pass it to some of the more posterior efferent vessels (Fig. 2).

These observations are directly antagonistic to those of other investigators on several points of anatomical detail and of function of some of the vessels. I have given above a statement of the general arrangement of the branching afferent vessels with reference to the nephridia; it will be evident from text-figure 2 that my results only agree in a general way with the statements

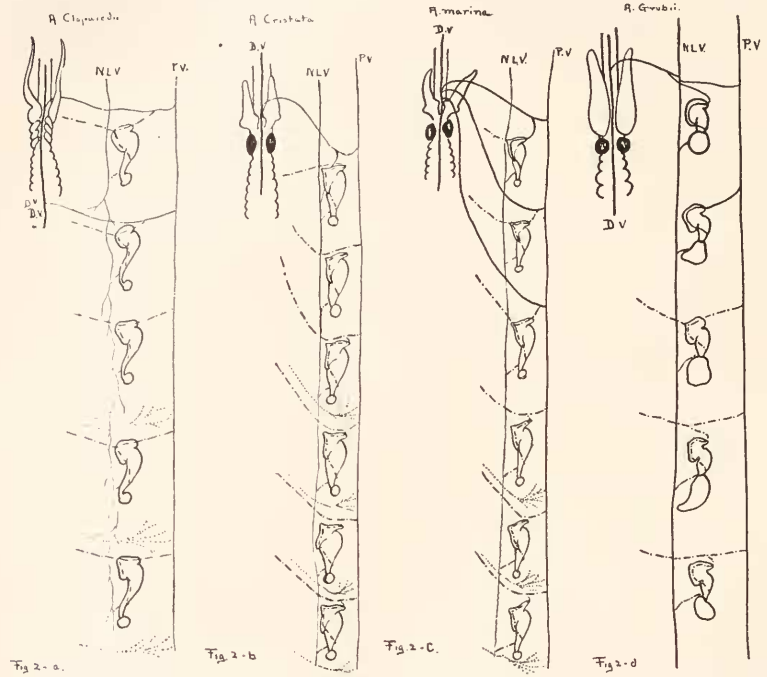

FIG. 2. Diagrams of the blood vessels supplying the nephridia in the several species of *Arenicola*, *a*, *A. Claparedii*, *b*, *A. cristata*, *c*, *A. marina*, *d*, *A. Grubii*; *d* will also serve as a diagram of the blood vessels of *A. ecaudata*. The form of the nephridia and of the digestive organs in the latter species, would be somewhat different but the arrangement of the blood vessels is the same as in *A. Grubii*. The dorsal vessel is shown by a solid line, thus ___. Afferent vessels arising from the ventral vessel are shown in broken lines, —.—.—. Efferent vessels connecting with the sub-intestinal blood vessel are shown in dotted lines, ... *D.V.*, dorsal vessel. *N.L.V.*, nephridial longitudinal vessel. *P.V.*, parietal blood vessel.

of previous authors. Thus in all nephridia of *A. Claparedii*, in some of *A. marina* and occasionally in *A. Grubii*, the afferent branch of the ventral vessel enters the *apex* of the funnel before it branches. In *A. cristata* one finds frequently, particularly in the somites containing the second and third nephridia, that the affer-

ent vessel gives off no branches to the nephridia, but the latter are supplied by a branch from the parietal.

The branch of the dorsal vessel to the second nephridium is small in *A. Claparedii*, but functional still. Gamble and Ashworth show and describe an afferent vessel to the first nephridium of *A. marina;* with this V. Willem disagrees, showing for this nephridium only a branch from the dorsal. In the majority of cases I find a branch from the ventral vessel also, thus agreeing with Gamble and Ashworth; in a few animals it is apparently wanting. I do not find, however, the efferent vessel from the fourth nephridium, in *A. marina*, to the subintestinal, which Gamble and Ashworth show but which V. Willem claimed was not present.

It is not true that the three main branches of the afferent vessel break up into capillaries; the one to the setal sac and gill does. The one to the integumentary vessels unites with them. The branch which enters the funnel continues as the gonadial vessel, runs peripherad to the nephridium and connects with the nephridial longitudinal, except in *A. Claparedii.*

The parietal and nephridial longitudinal vessels are distinct throughout the nephridial region in *A. Grubii*, *A. ecaudata* and *A. marina.* In *A. cristata*, the parietal is distinct but the nephridial longitudinal, while large at the level of the first nephridium, tapers posteriorly, becoming obscure back of the third nephridium. The parietal vessel is distinct the entire length of the nephridial region in *A. Claparedii.* The statement of Gamble and Ashworth that it is absent in this form is only explicable because they worked on preserved material; it is plainly evident in the living worm. The nephridial longitudinal, as a distinct vessel, is absent in this form except in the region of the first nephridium: its place is taken by a series of small connecting vessels, as if the gonadial vessel branched and some of its branches ran back to connect with those of the next posterior gonadial vessel.

In all cases the branches of the dorsal vessel running to the nephridia are afferent vessels. Gamble and Ashworth state that "The first nephridia of A. Grubii and A. ecaudata are *supplied* by a branch from the dorsal vessel" (the italics are mine), yet "The first three nephridia of A. marina, the first two of A. Clapa-

redii and the first of A. cristata *return* blood to the dorsal vessel." On *a priori* grounds, it would be strange to find the homologous blood vessels in closely related species carrying blood in opposite directions. I am certain that in *A. cristata* the blood flows from the dorsal vessel out to the parietal, the nephridial longitudinal vessel and to the nephridium, in this branch of the dorsal vessel. In this species the individuals are so large that in chloretonized specimens the direction of the blood movement is seen with comparative ease. S. E. Keith who has worked carefully for some time on the circulation of *A. cristata* gives me permission to quote her on this point as follows: "I have referred to these blood vessels in my own notes as the third pair of branches of the dorsal vessel. Gamble and Ashworth speak of the dorsal as receiving these branches, but the blood flows outward in them I am sure."

I have watched the blood-flow in *A. Claparedii;* the skin is frequently so transparent it may be seen without dissection. The flow is certainly away from the dorsal vessel in this species also. I am reasonably certain that such is the case in *A. marina*, in chloretonized specimens of which I have watched the blood movement. In this opinion I am supported by Willem who says of *marina*, "Il faut remarquer de plus, au point fonctionnel, que le sang circulant dans le tronc dorsal d'arriere en avant, le contenu des trois branches qui en emanent progresse dans une direction centrifuge."

Lillie makes the statement that "The vessel (segmental) begins its formation at the junction with the subintestinal blood vessel. Near its junction with the body wall the main vessel (segmental) gives off a branch (the nephrostomial vessel) which curves back, passes inward and barkward along the dorsal lip of the nephrostome." From this quotation it is evident that Lillie traces the afferent nephridial vessel to the subintestinal blood vessel, while, as stated above, I trace it, in agreement with Gamble and Ashworth and other observers, to the ventral vessel. The contradiction is only verbal, not real, for Lillie, in the explanation of his figures, labels this subintestinal vessel "the subintestinal *or* ventral vessel," a usage which he gets from oligochæt anatomy and which is incorrect here. Lillie finds that in *Arenicola* as in

other *Polychæta*, (see Fraipont on *Poygordius*, for instance), as well as in the *Oligochæta* (Wilson on *Lumbricus*), the first vessel to arise as a differentiation of the mesoderm, is the ventral vessel. Wilson says "The first vessel to appear (in *Lumbricus*) is the ventral or subintestinal." What the relation of this first vessel genenally is to the development of the circum-intestinal network in the polychæts is an unsettled point. (See Edward Myer's "Studien über den Körperbau der Anneliden," III., p. 464.) Recently Schiller writes as follows: "Nur bezuglich Blutsinus und Darmgefäsznetz ist mann bei *Arenicola Grubii* noch nicht ins klare gekommen, welches von den beider das primäre sei, da verscheidene Autoren ganz verscheidener Ansicht daruber sind. Die einer behaupten dasz zuerst der Sinus auftrete und sekundär sich in ein Netz auflöse: die anderen bezeichnen den Blutsinus als ein Produkt des zusammengeschmolzen Darmgefäsznetzes." He does not care to express an opinion on the subject although of the growing posterior region of *A. Grubii* he says: "Im Darmepithel keine besonderen Zellen vorkommen, die Antrit an der Bildung eines Sinus nehmen könnten." In considering der Blutsinus, Darmgefäsznetz und Subintestinalgefäsz he says: "Diese drei Gebilde werden hier zusammen als anatomisch und entwickelungsgeschichtlich zusammengehörende Elemente."

I have taken pains to quote in order to show that in spite of the difference of opinion regarding the origin of the network of intestinal vessels there is no tendency to include the ventral vessel in *Arenicola* or other polychæts as a part of this network which does, however, include the subintestinals. In *A. marina* we know (Benham, 1893) that the subintestinal vessels and the gastric longitudinals develop as the result of a fusion of the walls of the circular vessels of the alimentary canal. While in the oligochæts the subintestinal vessels split off from the ventral vessel. (Beddard, F. E., "Monograph of the Oligochæta," p. 70.) The term subintestinal vessel is an incorrect term for the vessels so named in *Arenicola*. Lillie should not have used the term subintestinal when he meant ventral, as these two terms are not interchangeable in *Arenicola*. The ventral vessel or subintestinal vessel of *Lumbricus* — they remain united in this form — is not homologous to the ventral plus the subintestinal vessels of *Arenicola*.

There is need of amendment in the description and figures of the form of the nephridia of the genus. The funnels, in particular, are much more symmetrical, in some species, than they have heretofore been shown. The usual technique is at fault in distorting them. The funnels are attached to the oblique muscles in *A. Claparedii*, *A. cristata* and *A. marina*. When the worm is opened and pinned out for dissection, the strain on these oblique muscles or on the connecting blood vessels of the other species, pulls the funnels throughly out of shape. It is, therefore, well to stupefy the worm by adding, drop by drop, seventy per cent. alcohol to the smallest quantity of sea water that will cover the worm in a long narrow dish. Then when the muscular walls are relaxed, the body cavity is injected by means of a hypodermic syringe or a fine pipette, until the walls are well distended with

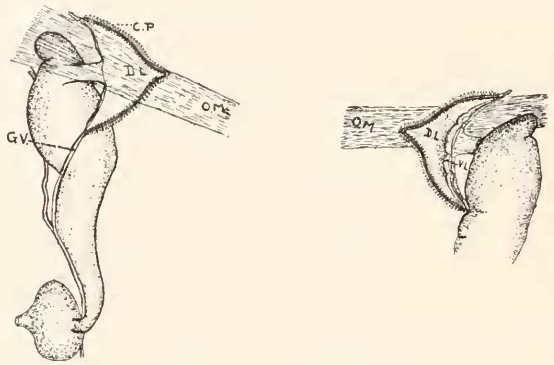

FIG. 3. Dorsal view of the second left nephridium of *Arenicola cristata*, × 3. *C.P.*, ciliated plates. *D.L.*, dorsal lip. *G.V.*, gonadial blood vessel. *O.M.*, oblique muscle.

FIG. 4. Ventral view of the anterior portion of second left nephridium of *Arenicola cristata*, × 3. *D.L.*, dorsal lip. *V.L.*, ventral lip.

the fixing agent. (Kopsch fluid answers well.) The whole worm is then placed in the fixing fluid. The nephridia are thus hardened without distortion and when dissected out later show their proper form.

The figures of the funnels or entire nephridia of each of the species are shown in the accompanying figures. The funnel of *A. cristata* (Figs. 3 and 4) is the most complicated and at the same time the most symmetrical in the genus. It is flattened, with a broadly sagittate or hastate form. Its dorsal lip,

which is attached by its outer surface to the oblique muscle, forms the point of the arrow. The ventral edge of this lip is set with from thirty to sixty ciliated plates which stand nearly at right angles both to the plane of the lip and to its edge. A loop of the blood vessel which traverses the dorsal lip runs up into each plate. The ventral lip is bow-shaped and divided into three segments like the handle and ends of a bow; the convexity of the bow turns toward the apex of the dorsal lip. The opening of the nephrostome is a long narrow slit between the base of the triangular dorsal lip and the bow-shaped ventral lip; the opening leads into the rapidly narrowing throat. The hastate funnel is held to the body by a short slender haft; the body of the nephridium, the glandular portion, is club-shaped; the funnel attaches to its side near the larger end, the axis of the

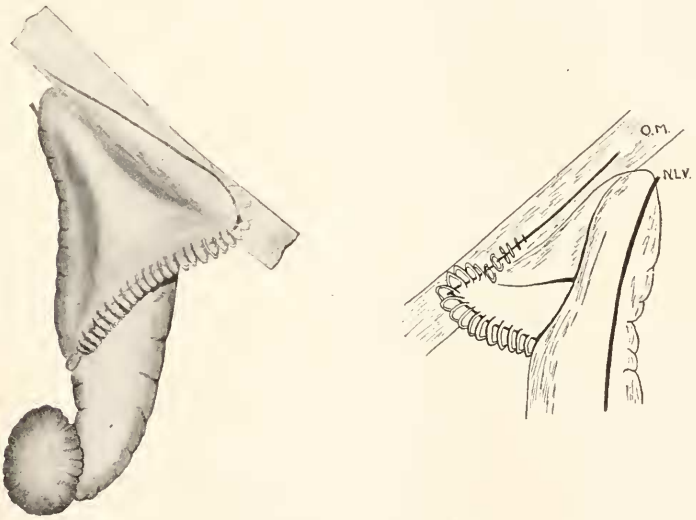

FIG. 5. Dorsal view of a nephridium of *A. marina*, × 15.

FIG. 6. Ventral view of the anterior end of the nephridium of *Arenicola marina*, × 15. *N.L.V.*, nephridial longitudinal blood vessel. *O.M.*, oblique muscle.

funnel being nearly at right angles to the longitudinal axis of the body. The body of the nephridium joins the roughly spherical bladder at the smaller end of the body, the handle of the club. The bladder opens to the exterior on its peripheral side by a very short duct leading to the nephridiopore.

The nephridium of *A. marina* approaches, at times, quite nea

to the form of that of *A. cristata;* but usually it is quite different from it (Figs. 5 and 6). The general shape of the body and of the bladder is nearly the same as in the nephridium of *A. cristata*, although in *A. marina* the body is more frequently curved while that of *A. cristata* is straight. The funnel shows great variation and often departs widely from the type of *A. cristata*. It is a flattened flap having roughly the shape of an equilateral triangle. At one angle the funnel opens into the anterior end of the body of the nephridium; along the opposite side lies the nephrostome. One of the sides adjacent to the neck is attached to the oblique muscle; the other adheres to the margin of the body. The axis of the funnel forms, therefore, an angle of only thirty or so degrees with the axis of the body. The dorsal lip is straight or slightly concave; it is set with twenty five or thirty somewhat lanceolate ciliated plates, each of which is supplied with a loop of the blood vessel. The ventral lip, Fig. 6, is regularly concave.

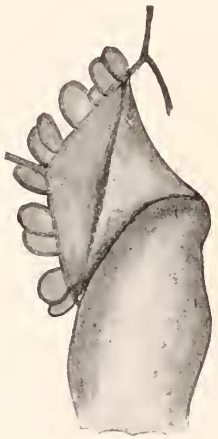

FIG. 7. Ventral view of the anterior portion of the third left nephridium of *Arenicola Claparedii*, × 30.

FIG. 8. Dorsal view of the nephridium of *Arenicola Claparedii*, × 15.

The ciliated plates which run along the edge of the dorsal lip, tend to continue along the blood vessel past the angle which the dorsal lip makes with the side of the funnel that attaches to the oblique muscle. This blood vessel runs along the muscle nearly parallel with the edge of the funnel. There are from ten to twenty of these plates; those along the blood vessel on the muscle

diminish in size. When occasionally there are an unusually large number of them along the blood vessel beyond the angle, the angle of the funnel adjacent to the muscle tends to become the apex of the funnel as in *A. cristata*, and the mouth shifts so as to more nearly face this angle instead of a side. In such cases the throat of the funnel opens into the body some distance back of the anterior end of the latter.

The funnel of the nephridium of *A. Claparedii* (Figs. 7 and 8) is least complicated. If we imagine a simple funnel form with short stem to be flattened and to have one lip pulled out into a triangular protrusion, we may gain a clear idea of the funnel of this species. The apex of the dorsal lip is broadly obtuse; the ventral lip is straight. The dorsal lip is set with the ciliated plates characteristic of the *marina* section of the genus which, as Gamble and Ashworth point out, includes the species *Claparedii*, *cristata*

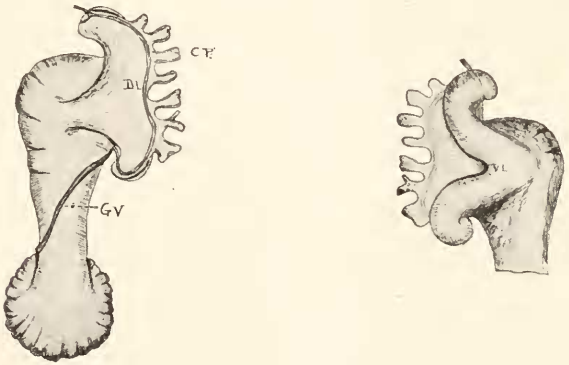

FIG. 9. Dorsal view of the first left nephridium of *Arenicola ecaudata*, × 15 *C.P.*, ciliated processes. *D.L.*, dorsal lip. *G.V.*, gonadial blood vessel.

FIG. 10. Ventral view of the anterior portion of nephridium (first left) of *A. ecaudata*, × 15. *V.L.*, ventral lip.

and *marina*. There are ten or twelve of these plates in *A. Claparedii*. The funnel is at the anterior end of the body and its axis is also at right angles to the axis of the body of the nephridium.

The funnel of the nephridium of *A. ecaudata* (Figs. 9 and 10) is reniform in outline with revolute ends as seen from the ventral face. It also is flattened; the dorsal lip is slightly concave, the ventral lip is deeply indented. The dorsal lip is provided with ten to thirty blunt, at times much branched, finger-like processes;

these are covered with cilia, and within each is a blood sinus instead of a loop of the blood-vessel. The throat of the funnel is relatively wide; the funnel attaches at the end of the body and usually has the customary position with its axis at right angles to the long axis of the nephridial body. Not infrequently however, the axis of the funnel is continuous with the axis of the body and we have a simple, unbent, tubular nephridium (Fig. 11). It is interesting to find such variation in this species for it makes evident how the more aberrant forms of the nephridium, such as occur in *A. marina*, are derived from a simple tubular type; just as a paper tube may be bent with its end at right angles to its major axis.

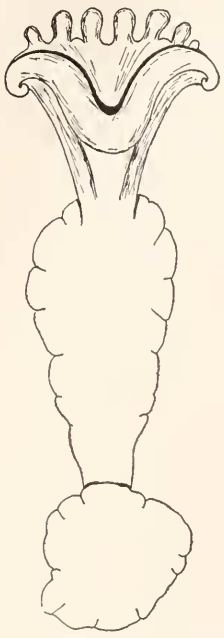

FIG. 11. An occasional form of the nephridium of *Arenicola ecaudata*, × 20.

A. Grubii (Figs. 12 and 13) possesses the same type of funnel as *A. ecaudata*. It is flattened; the dorsal lip is semicircular, the ventral lip trilobate, with a small median lobe and large lateral ones, thus making this lip deeply notched. Ten or twelve blunt, digitate, ciliated processes attach to the dorsal lip; these are often branched and are provided with the blood sinus. The funnel attaches to the body at some distance from the anterior end and its axis is at right angles to the axis of the body. The bladder of the nephridium of this species is usually expanded: it is capable of equally wide expansion in *A. ecaudata* but is more often found contracted. In the other species, the bladder is not so distensible although it is relatively large at times, especially when filled with eggs or sperm about to be discharged through the nephridiopore.

I have collected *A. cristata* and *A. marina* near Woods Hole, Mass., *A. marina*, *A. Grubii* and *A. ecaudata* in the bay at Plymouth, England, and have studied fresh *A. Claparedii* and *A. Grubii* at Naples. I wish to express my sincere thanks to the directors of the biological stations at these several places, who

have kindly placed at my disposal, material and facilities for the work, and to the Carnegie Institution whose table at Naples it was my pleasure to occupy.

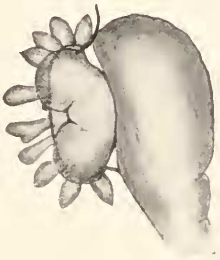

FIG. 12. The nephridium of *Arenicola Grubii*, × 15, dorsal view. The outline of the ventral lip of the funnel is shown in dotted line.

FIG. 13. Ventral view of anterior portion of the third left nephridium of *A. Grubii*, × 20.

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SOME OBSERVATIONS ON THE HABITS OF PECTEN DISLOCATUS.

B. H. GRAVE.

With the purpose of studying the habits of the scallop, *Pecten dislocatus*, I collected many young specimens ranging from two to ten millimeters in length and placed them in small glass aquaria in the laboratory.[1] They were found in the harbor at Beaufort, N. C., well above the muddy bottom, clinging to eel grass. They were usually attached by several strands of hyaline byssal threads, which were exceedingly strong and elastic.

Although the *Pecten* is so generally known as to make a detailed description of its anatomy superfluous, yet a brief description of certain parts is deemed necessary. For a more detailed study of the anatomy, reference can be made to a paper, by G. A. Drew, on "The Habits and Anatomy of the Giant Scallop."

By reference to Figs. 1 and 2, it may be seen that the shell is rounded and eared. The ears make possible the long, straight hinge line, which extends along their upper borders to their extremities. The right valve is slightly more convex than the left, and near the anterior[2] ear, it has a deep notch. This one feature mars the symmetry of the valves. Between the valves and just beneath the hinge ligament, there is a pad of cartilage-like substance, which is compressed when the valves are closed. It serves to open them quickly when the adductor relaxes (Fig. 3).

The form of the shell and the structure and arrangement of the soft parts within, adapt *Pecten* to the swimming habit. It swims by opening and closing the valves in rapid succession. By varying the position of the mantle so as to control the direction of

[1] Through the courtesy of Hon. Geo. M. Bowers, U. S. Commissioner of Fish and Fisheries, I had the privilege of occupying a table in the Fisheries Laboratory, at Beaufort, N. C., for two months during the summer of 1908. For this privilege and for many kindnesses shown me by the Director, Henry D. Aller, I am glad to express appreciation.

[2] The hinge line is here considered dorsal for convenience in description, although it does not represent the true dorsal of the animal.

the currents of water expelled from the mantle chamber, it is enabled to swim either forwards or backwards, although it usually swims with the opening of the valves directed forwards.

A perfectly symmetrical shell is the form best adapted to swimming, and the presence of any irregularity in it, such as that just mentioned in the pecten, is to be explained either as an adaptation to habits other than swimming, or as a structure, inherited from an ancestral form, and not as yet obliterated through adaptation to the swimming habit.

Although adult specimens were kept in aquaria all summer, no method of locomotion other than smimming was noted, and no clue was gained as to habits which would in any way explain the function of the notch in the right valve. They neither attached themselves by a byssus, nor used the foot for locomotion. Young specimens, however, showed much more activity than the adults, and some observations on their habits are recorded in the following pages.

Concerning the function of the notch in a related species, *Pecten tenuicostatus*, Dr. Drew writes as follows: "I have been unable to satisfy myself as to the function performed by this notch. The sense tentacles on the mantle margin, opposite the notch, are somewhat longer than those adjacent, but I have been unable to determine that they have a special function or that they are especially advantageously placed."[1]

The Sense of Position.

The *Pecten* lies habitually upon the right valve and if placed upon the left, immediately turns over. When lying upon the left valve, it seems to feel the same sort of discomfort which a frog, or other animal with well developed balancing organs, feels when placed upon its back. However, after turning them over repeatedly, they sometimes remained resting on the left valve for several minutes.

The Asymmetry of the Valves.

When dropped through a considerable depth of water, *Pectens* settle about as frequently upon the left valve as upon the right.

[1] *The University of Maine Studies*, No. 6, September, 1906, p. 7.

The slight flatness of the left valve does not serve to make them settle always upon the same side.

The Functions of the Foot.

The foot lies just opposite the notch in the right valve. It appears to be functionless in adult specimens, or rarely used by them, but is made use of to good advantage by the young. Specimens were often seen to extend the foot anteriorly to a remarkable distance, attach it at the tip to the bottom and then, by a powerful contraction, draw the body forwards to the point of attachment. The foot is cylindrical and seems very small to carry such a load; and frequently, after it has been extended and attached, the valves are opened and clapped together, at the same time the foot contracts, the body thus being drawn forward with much less strain upon that organ. This method of locomotion is a combination of swimming and creeping. The force of the current of water expelled from the mantle chamber serves to raise the body and propel it forwards as far as the attached foot will permit. At the same time, the foot contracts and the body lands close to the point of attachment. When this method of locomotion is used, the foot, instead of being extended directly outwards, anteriorly, is usually directed more ventrally, so that the point of attachment is more nearly in line with the force exerted by the swimming movement. Except for the notch in the right valve, this sort of performance would not be possible, because by the closure of the valves, the foot would be crushed.

The foot is, also, frequently used in turning the body over, when placed upon the left valve; it is extended anteriorly from the body and attached; the valves are opened and clapped together vigorously; the body, as a result, is raised and shot forwards, but the weight of the foot and the resisting pull from its attachment cause it to swing over upon the foot as a pivot, the scallop landing upon the other valve, having turned through an arc of 180 degrees.

The above method of turning over, is usually, if not quite universally, used by specimens when placed upon the left side for the first time. After a little handling, however, they become much more irritable, seeming to be excited, and at such times, they

manage to right themselves by one of three methods: Sometimes without extending the foot, they open the valves and clap them together. After one or several trials, the body turns over upon the hinge line as a pivot. The mantle must have played a part in this by expelling currents of water in a direction such as to cause the body to turn over.

At other times, a position on the right valve is gained by one or more short swims, the method being continued until the body comes to rest on the right side. They usually manage to alight upon the right valve after a few trials, and then they become quiet.

The Byssus.

When specimens are allowed to lie undisturbed upon the right valve, they usually become attached by numerous strands of strong byssal threads. A short time only is required for this to take place. They frequently become firmly fixed in from two to five minutes and the threads are sufficiently strong to support a weight several times that of the body of the *Pecten*. The byssal threads pass through the notch in the right valve directly to the support below. They adhere, to some extent, to the shell where they come into contact with it.

So long as specimens are kept lying upon the left valve, they cannot, or do not, attach themselves by the byssus. Since the byssal gland lies at the base of the foot, it is possible that the notch, in the shell opposite it, is a structural adaptation directly correlated with the function of the byssus. At any rate, because the byssal threads extend through the notch, in place of over the edge of the shell, the pull has less tendency to tilt the body than would be the case if no notch were present.

In order for the byssus to become attached to the bottom, it is not necessary for the valves of the shell to be opened, since the attachment of the byssus is frequently accomplished while they are closed. The notch in the shell is sufficiently large to allow the extension of the foot to the support during the process of attaching the byssus. It seems that Dr. Drew has observed this process in individuals of *Pecten irradians*, to quote:

"An individual of *Pecten irradians* placed in a glass dish of sea water will sometimes protrude its foot from the shell, apply it

closely to the bottom of the dish and after a short time, slowly withdraw it, leaving a rather broad band of slightly yellowish material attached to the glass and connected with the foot by the byssal gland. This is not composed of small threads as in the mussels *mytilus* and *modiola*, but it may be sufficiently tough to support the weight of the animal, if, after a few minutes, the dish is carefully turned over."[1]

Summary.

By way of summary, therefore, it might be said concerning the function of the notch that it makes possible a much freer use of the foot and byssal gland, and is in some way connected with the function of these organs. Although many mollusks live in the mud, the fact that young *Pectens* do not is evidence that they do better out of it. The foot and byssus enable them to climb upon supports and maintain their position there. As they approach maturity, they assume more and more the swimming habit and the foot and byssus lose, to some extent or entirely, their functional activity. If these organs are not functional in full-grown *Pectens*, as seem probable, the notch is no longer of any value to them, although it is not obliterated.

The *Pecten* has the sense of position well developed.

Earlham College, Richmond, Indiana,
March 6, 1909.

[1] *The University of Maine Studies*, No. 6, September, 1906, p. 18.

Explanation of Plate I.

Fig. 1. Shows the left valve as it appears from surface view. It is not quite symmetrical.

Fig. 2. Shows the right valve as it appears from surface view. The prominent notch at the base of the anterior ear can be seen.

Fig. 3. Is a view of the inner surface of the right valve. The dotted line shows the position of the cartilage pad which aids in opening the valves.

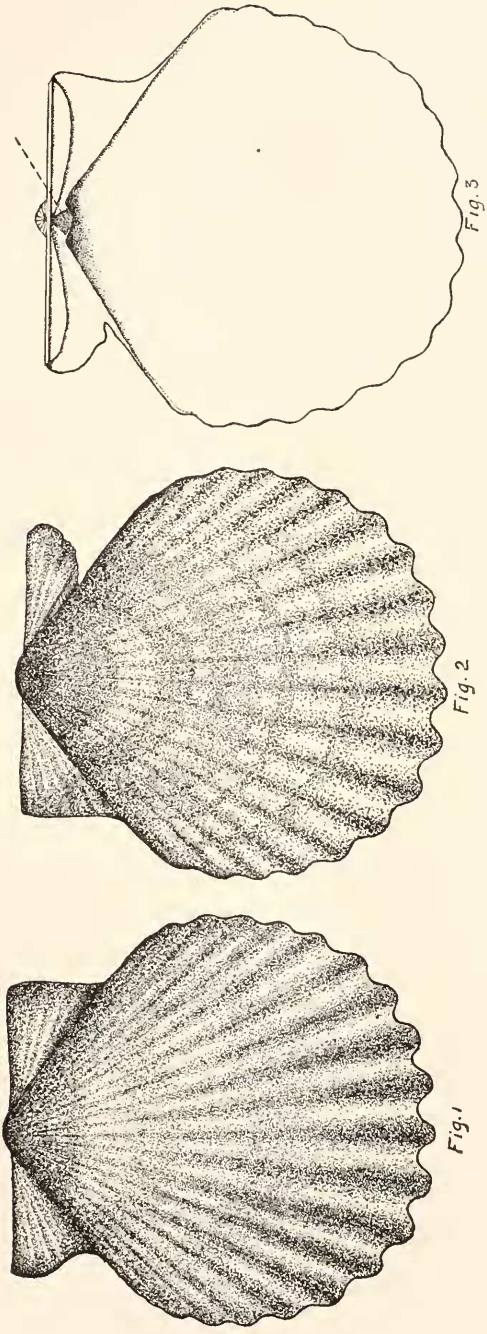
Fig. 3
Fig. 2
Fig. 1

THE DYNAMIC FACTOR IN REGENERATION.

T. H. MORGAN.

With the publication of the data here presented the series of experiments that I have carried out on *Tubularia* for several years may be considered as temporarily brought to a close. I take this opportunity therefore to sum up the evidence bearing on the problem of the formative factors of regeneration, as exhibited by this hydroid. In the course of my experiments tentative hypotheses have been proposed here and there that have at least served to suggest further experiments. The conflicting evidence sometimes inclined me towards one point of view, sometimes towards another; yet, all in all, the same general line of thought, if sometimes vague, can be traced through the attempts to analyze the results. It will be my endeavor here to bring more into the foreground those theoretical deductions that seem to me at present to be best in harmony with the experimental evidence.

That the dynamic factor in regeneration is not primarily the outcome of physiological movements of the animal, or of its parts, is made probable not only by many facts familiar to every student of regeneration, facts that show that the new part often develops under conditions where movement or function in this sense is absent, but also by the important experiments of Zeleny and of Stockard with the jelly-fish *Cassiopc.* They have shown that when one half of the disc is brought to rest by removing the sense organs (and scratching a barrier zone across the connecting ectoderm) the quiescent half regenerates as well and as rapidly as the half left pulsating during the period of regeneration.

Since every part of the stem of *Tubularia* is capable of producing a hydranth the inhibition of basal development is obviously due to the presence of a hydranth or of a developing hydranth at the oral end. Two alternative chemico-materialistic explanations have been suggested for cases like this. (*A*) The oral hydranth may use up some materials necessary for the formation

of a basal hydranth. (*B*) The hydranth may produce some materials that inhibit the development of other hydranths from the remainder of the piece; hence the inhibition, as long as a hydranth is present or developing. On first thought these alternatives would seem to cover the only possible ways in which the problem of regeneration may be presented — at least as long as the problem is confined to purely physiological actions of a chemical order. There are, however, not a few considerations indicating that the fundamental interpretation may lie in a different conception of the problem. I shall try here to emphasize this other point of view without attempting to develop it into a theory of regeneration. At most we may hope at the present time to find in the facts some indication of the nature of the problem if not its entire elucidation.

A number of experiments have been made that seem to indicate that the temporary inhibition of the development of the basal hydranth in *Tubularia* is not the result either of the using up of materials by the oral hydranth, or of the setting free of inhibitory stuff. The simultaneous development of hydranths at both ends of a piece, which frequently occurs in short pieces, is a case in point. Both ends develop at the same rate as when a single hydranth develops, and not half as fast as the hypothesis demands. Again the development of a basal hydranth does not appear to inhibit the oral development as we should expect if the result were dependent simply on the presence of materials in the stem. Some experiments of MacCallum's with plants have an important bearing on this point. If the terminal bud of the bean is removed, the buds in the axils of the cotyledons develop. But if the activity of the terminal bud is simply lessened by inclosing that part in an atmosphere of hydrogen, the basal buds do not develop. Hence the result is due not to activity of the terminal end, but to its presence or absence. In a different way the same fact is brought out. A piece of willow stem is cut off, its middle third is inclosed in a tube filled with moist air, so that the buds in this part are encouraged to begin their development; the dry air retarding the development of those outside. After the middle buds have unfolded, the entire piece is inclosed in a moist chamber, when the more apical buds sprout forth, while none

of the buds basal to the middle region develop. The presence of growing shoots in the middle of the piece does not inhibit the apical buds from developing, if external conditions are supplied favorable to their growth, but the basal buds are inhibited by the presence of shoots on the more distal parts. These facts are incompatible with the assumption that the results are due to the presence of materials used up by those parts that develop first to the exclusion of other parts. They also show that the alternative view is untenable, for, the presence of growing shoots in the middle of the piece is not antagonistic to the development of shoots in other regions provided those regions are situated more distally.

In the case of *Tubularia*, it is more difficult to present convincing evidence that distal hydranths do not produce materials inhibiting the development of basal hydranths, improbable as such an interpretation may now seem. But the fact that basal hydranths do develop after the oral hydranths have formed may seem to discredit this view. Here, however, an apparent paradox is found. The experiments seem to show that when the oral hydranths develop, the basal hydranths are retarded in development, but they do develop later, and the results also show that if both start simultaneously both develop at the normal rate. The paradox is due, I think, to two antagonistic factors at work at the same time. Admitting that the oral development tends to inhibit the beginning of basal development, we also find that if other influences suffice to start both simultaneously, the on-rush, so to speak, of the process once begun changes the conditions that tended to prevent the starting. Strange as this seems it is little more than a statement of the facts. The same results may be put in a somewhat different way. A cut end being present, whether oral or basal — the conditions that call forth hydranth formation are given. Experiments show that the oral end tends to develop first, its development acts as a partial inhibition of the basal hydranth-formation. If this influence is strong enough the basal development is temporarily held in abeyance, but if not the inhibition is overcome. Once overcome, the formative influences do not check the further action of the basal end. In this connection it is curious to note that small oral pieces produce simultaneous hydranths more often than larger

pieces. The interpretation of this seems to be that the tendency to produce hydranths, both oral and basal, is stronger near the distal end and decreases basally. In short pieces the sensitiveness of the two ends to those influences that call forth the hydranth is so great that both ends develop simultaneously or nearly so, hence the oral end has not time to get a sufficient start over the basal to stop its development. It should be noted in passing that it is probable that the influence preventing basal development is not only the oral development, but a direction-factor present in the stem at all times. This factor we call polarity. The interesting point is that this factor seems to be more capable of inhibiting basal hydranth formation when an oral end is developing than when such development has not yet begun. The basal development, however, does not appear to delay the oral process. It is acting against the polarization and its influence is less felt throughout the stem, as experiments by Stevens and myself have shown. These considerations lead, I think, to the view that the essence of our problem lies in that peculiarity of the piece that we designate its polarity, and not in the absence or presence of formative substances in Sach's sense.

If our analysis is correct, we are led to look upon living material as possessed of a certain formative principle that has so to speak a "sense of direction." The next step will be to study the nature of this principle and see what properties we are justified in ascribing to it; for while it may be beyond our powers at present to state precisely the nature of the directive principle, we may at least be enabled to work out its manifestations. Some of these manifestations become apparent in the study of the regeneration of *Tubularia*. One of its most striking modes of action is seen in the inhibition of basal-hydranth formation. Most interesting is the result that its action becomes intensified by developmental processes going on at the oral end, as shown by the fact that if the oral development is suppressed by tying that end, the basal development is much accelerated. It is accelerated in the sense that basal hydranths more often develop at once than when both ends are open, but not in the sense that the basal development is faster than when this end also gets as early a start as the oral end. In other words, there is no speed-

ing up of hydranth formation as such, but the initial inhibition is overcome.

The special problem with which this paper deals is the nature of what takes place at the basal ends when the oral end is kept open and when it is tied. Is the retardation of such a kind that a slower process of development is going on at the basal end while the oral end is developing, or does the basal end not really begin to develop until the oral end has formed its polyps. If so, what gives it its start later? The following experiments were devised to study these questions.

Experiment I. — The purpose of the experiment was to determine whether when both oral and basal ends of a piece are left open constructive changes are slowly going on at the basal end. Some pieces were cut off and left open (*A*); later other pieces were cut off and the oral ends tied (*B*) and at the same time the oral ends of (*A*) were tied. It was found that the basal ends of the (*A*) pieces did not develop faster than those of the (*B*) pieces, showing that the changes at the basal end of (*A*) are not progressing, but are held in check by the developing oral hydranth.

Control I. — In some pieces the old hydranths were left intact and the pieces cut off. No basal hydranths began to develop until the old heads began to be absorbed. The presence of the old heads inhibited the development of the basal hydranths until the heads had degenerated when the latter appeared.

Experiment II. — In order to find out whether, when the oral end is tied, changes take place throughout the piece that tend to make more rapid the development of basal hydranths, or whether these changes are localized at the basal end where the new hydranth develops, the following experiment was tried. The oral ends of many pieces of the same length were tied. Then after several hours' interval differing in several experiments, the basal end was cut off, (*a*) just inside of the area that would form the basal hydranth, (*b*) in the middle of the piece, (*c*) just below the ligature. In general the development of the basal hydranth was delayed as compared with control pieces tied but not cut off at the basal end; the delay was the greater the further removed the cut from the basal end, despite the fact that oral levels tend to regenerate faster than more basal levels. The differences

are more apparent the longer the time that elapses before the basal pieces are removed. The differences are not very marked at the different levels indicating perhaps that changes take place throughout the piece and not only at the basal end although more pronounced in the latter. The different levels of the cuts make it difficult to ascribe the results solely to the general changes in the piece, for the more orally situated cut ends have an advantage in level as other experiments have shown.

Experiment III. — Pieces were cut off at the same oral levels. After 23 hours the hydranth region at the oral end was cut off of some pieces (*A*), others were cut in two in the middle of the piece (*B*), and for a control some pieces were left as before (*C*). A slight retardation occurred after another 12 hours in (*A*), less in (*B*) as compared with (*C*). Removal of the hydranth forming region after 23 hours causes delay but the delay is not so much as though a new hydranth had developed at the new cut, showing that changes directed towards hydranth formation are going on not only in the region where the hydranth will develop but at more basal levels as well.

Experiment IV. — This experiment was like the last, except that the basal ends of all the pieces were tied, thus preventing the basal end from exerting any influence on the result. Other experiments had shown, however, that the basal development, even if it occurs, has apparently no retarding influence on the oral development. The results, as was to be expected, were the same as in the last experiment. It is interesting to note that in both the influence of the cutting causes a greater delay in the first appearance of the primordia of the hydranth than on their later development; for later the differences seem to be less than at first. This may be due to an acceleration extending throughout the whole time that is more effective after a beginning has been made than before the start.

Experiment V. — Some previous experiments had left undecided the question whether, when the oral end is left open for several hours and is then tied off, the basal development is more rapid than when the oral end is tied at once. If such an acceleration really occurs it might seem to indicate that changes take place in the oral end that produce accelerating materials even for the

basal ends. I was particularly anxious to settle this point definitely, for obviously, if such acceleration could be proved, it would furnish evidence in favor of a chemical process, especially since other experiments had seemed to show that the basal end does not begin its development when both ends are left open. I have carried out rather an extensive series of experiments that give, I think, a definite answer to the question.

When pieces are left open at both ends from four to nine hours, and are then tied at the oral end, the basal development is slightly retarded as compared with its development in pieces tied at once. There is little evidence in favor of the view that the later tied pieces can make good the loss of four to nine hours, and of course they can not catch up if a longer time elapses. Whether they may do so in later stages is more difficult to decide, but this does not concern the main point here raised.

Individual differences in rate, differences in stems, and uncontrollable differences in level tend to obscure results that depend on only four, six, and nine hours differences in start. The above statement holds, therefore, only for average results. There was found no evidence in favor of actual acceleration, whether there is some relative acceleration is difficult to decide. If the hydranths do not develop promptly *i. e.*, if a long time elapses between the tying and the appearance of hydranths, the initial differences of a few hours may be lost.

Experiment VI. — Another attempt was made to see whether changes take place in the piece as a whole, after it is cut off, that make more rapid the development of an *oral* hydranth when a new cut is made.

Pieces were removed and after four hours somewhat more than the oral hydranth region was cut off. In some cases the newly cut ends developed as fast as did the hydranth in the small pieces cut off, but the latter may have been retarded by the operation or by the smallness of the pieces ; yet in some cases the development of the newly cut ends was as rapid as in control uncut pieces. This result indicates that changes take place in the pieces behind the actual region of hydranth formation that lead toward the development of a hydranth.

Experiment VII. — In this case pieces first cut off were after

ten hours cut in two in the middle. Comparing the rates of development of the oral ends of the oral halves with that of the oral ends of the basal halves it appears that the latter are slower, but there is evidence that the retardation may be somewhat less than the ten hours difference in initial start. The basal halves in this experiment are also somewhat behind the basal halves in the last experiment, which seems to show that the general changes in the excised pieces that go towards oral development decrease from the cut ends inwards.

It has not seemed necessary to give the details on which these general conclusions are based. The nature of the case makes it difficult to obtain results as definite as one might wish, despite the precautions that were undertaken to make the conditions as uniform as possible. The general conclusion that changes take place in the piece as a whole, after its removal, that are in the direction of hydranth formation, seems fairly certain. Less certain perhaps is the evidence to show that when the oral end is tied similar changes take place in the pieces that accelerate basal development in regions beyond the hydranth forming region, but this conclusion too is, I think, quite probable. The nature of these changes is not revealed.

The Dynamic Factor in Egg-Development.

Students of the processes of regeneration have without exception made use of the term polarity to express a directive factor observable in their results, and to this factor is sometimes ascribed an active rôle as a controlling influence, at other times the term is used descriptively merely as a statement that the new structures are directed in the same way as the part removed. In both respects the word has been useful, however vague our conception of what polarity may be. Our analysis of the process has now gone sufficiently far, I think, to justify us in an attempt to come to closer quarters with the term. Without reviewing the opinions that have been expressed as to the nature of polarity, I shall try to contrast two views of its nature that seem to me to represent the two main lines that speculative thought has followed. It should be noted that the term is used equally by students of embryology and by students of regeneration. The former

finds axial relations in the egg — polarity, bilaterality, radial symmetry, etc.; the latter finds the new organs regenerated in definite relations to the old. To some observers the distribution or the stratification of the materials of the egg, has seemed a sufficient basis for the results referred to under the term polarity; to others it has seemed more probable that there exists in the egg an arrangement or structure that has axial relations from which result not only the depositions of the formed materials but also the nature of the action of the parts. Polarity is from this latter point of view not simply a passive structure, but a relation of the parts that directs the shifting series of changes that we call development. At one time one of these views has seemed more probable; at other times the other. The history of modern experimental embryology and regeneration shows the influence that these views have had on those who have followed the new work. In a general way the two views may be classed as the materialistic or chemical and the dynamic or physical conceptions of the developmental process. At present it seems to the writer that the evidence has been steadily pointing to the second of these contrasting views as the more probable. As far as the egg is concerned, the recent experimental work goes to show that the visible inclusions of the protoplasm (yolk, oil and other granules perhaps) are not the fundamental causes of the formative processes, although they may be needed in certain regions to carry out the future development of the structures that there appear. In regard to regeneration it has been evident for some time, that the specification or the differentiation (with its concomitant products), cannot be unreservedly utilized as a basis for an explanation of formative processes that take place. For example, if the gross materials or the differentiations of the head end of a planarian are the causes of that region being a head, it is inexplicable that when the head is removed it could regenerate a tail. There must be something else behind what we see that is responsible for the change that takes place. These and other considerations lead to the view that there exists a fundamental property of living matter that is the formative principle of development. On two former occasions, when attempting to analyze the results of regeneration in *Tubularia*, the author tried to account for the re-

sults of polarity on the basis of a stratification of the materials. Influenced at the time by recent results in experimental embryology that seemed to show that visible substances of different kinds in the egg are really responsible for the development of its parts, the same idea was applied to the problem of regeneration, despite the fact that I had on more than one occasion rejected the hypothesis of formative stuffs, in Sach's sense, as sufficient to account for the facts of regeneration. Yet a careful reading of the papers here referred to will show that I still held, though perhaps not always consistently, to the conception that back of these differentiated materials lay the real differentiating factors.[1] It now seems to me that the evidence, which at that time seemed so strongly to favor the idea of the importance of the grosser materials of the egg, is insufficient to establish its case, and that the important factors of development are dynamic properties of the bioplasm, rather than the formed products of the egg, or of the differentiated products of the adult animal. This statement does not mean that the visible products in the egg play no rôle in development. The evidence still shows that they may do so, but their rôle seems to be secondary, not primary.

The interrelation of the parts seems to be one of the most evident expressions of the fundamental formative influences. Several years ago a consideration of a number of results in regeneration led me to state that this relation might be expressed as a sort of tension. This view has been objected to on the ground that it does not appear to explain the matter any better than before. In a moment of doubt and in order to give the

[1] One further word of explanation. The rate of hydranth formation varies with the distance of the cut end from the original hydranth. I have spoken of this difference in rate as explicable on the assumption of the hydranth-forming materials decreasing toward the base, *i. e.*, away from the hydranth. It was unfortunate to have used the term hydranth materials, although I made sufficiently clear in the text that I did not mean to invoke the stuff-hypothesis in this connection. It is not entirely clear on what the difference in rate depends; most probably on the stem being less specialized as a store-house of food substance nearer the hydranth; probably also on some difference connected with the thickness of the walls with which the specialization may also be connected; possibly neither of these but some more fundamental characteristic is responsible for differences in rate. In any case it is not obvious that there is any connection between this difference in rate and the polarity of the piece. The latter is the same for all levels — the time it takes the piece to be remodelled seems to be referable to something else.

statement a meaning for those who believe no suggestion to be of value unless it refer the problem to ordinary properties of inorganic bodies, I suggested that osmotic pressure might be the cause of the tension differences in the parts. This was an unnecessary concession. The behavior of fluid crystals (according to Lehmann) shows that the formative changes can be accounted for on the basis of a tension exhibited by the molecules of the substance of the crystal. While the organism may not be put down as a fluid crystal, still we see that physical properties other than osmotic pressure and surface tension may play an all-important rôle in form-changes. It may be that a similar property is the cause of the formative changes in the organism. In any case, the facts that I had in mind suggested that tension of some sort is an important dynamic factor in development, perhaps *the* important factor. The facts still seem to me to indicate some such relation between the parts, and no one regrets more than I that we cannot "explain" the results even if my suggestion prove to be in the right direction.

Still later a consideration of certain facts of development led to the suggestion that two known properties of the organism — contractility and irritability — also play a very important rôle in embryonic and regenerative development. I shall not attempt to review here the argument which led to this point of view. How far and in what sense contractility and irritability are better expressions of the tension hypothesis, it is not easy to state. So far as contractility is concerned Lehmann's recent important paper on "Scheinbarlebende Kristalle"[1] shows the possibility at least of referring this property also to a condition of molecular tension. We are still too ignorant of the physical basis of irritability to make speculations in this direction profitable, but it may be well not to lose sight of this property of living matter in our attempts to analyze further the problem of development.

Stereometry of the Bioplasm.

Polarity implies difference in one direction. Every student of regeneration knows that in all three dimensions of space the same factor is present. Polarity is therefore only a part of the problem,

[1] *Biol. Centralb.*, XXVIII., 1908.

and so far as it draws attention away from the whole problem it seems best to substitute the term stereometry.

Sufficient evidence has accumulated, I think, to show that stereometry has a dynamic side — in so far as it is a result of the molecular factors that determine the relations of the parts to each other. A question of fundamental importance here presents itself. If the formed substances at each level are the products of the bioplasm, must not the bioplasm itself be stratified in nearly the same sense? It was this idea that I had in mind when I wrote in 1906: "If we imagine a stereometric network as a part of the specialized structure, we must be prepared to admit that it changes at each level as the structure changes. Therefore it seems to me simpler to base our hypothesis of polarity on the difference in differentiation itself, and not on an imaginary polarized system associated with the living materials." But the point I overlooked was that there is no need to suppose that a heterogeneous network of bioplasm exists because the visible structure formed by it is different. The relation of the polarized material to the ends of the material (indeed to all its directions) suffices to account for the difference of level. In fact if the stereometry rests on a dynamic and not a statical relation of the parts this is the logical standpoint.

It has been suggested that irritability may be related to the dynamic factor of development.

The effects of irritability at any level may be realized through the chemical changes inaugurated. These chemical changes once started may, if enzymatic, thenceforward continue (unless checked by other chemical processes), independently of the factor that set them going.

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BIOLOGICAL BULLETIN

THE REGULATORY CHANGE OF SHAPE IN PLANARIA DOROTOCEPHALA.

C. M. CHILD.

The recent discovery of *Planaria dorotocephala* Woodworth (Woodworth, '97) in very large numbers, near Chicago, has made it possible for me to use this form for extensive series of experiments. The species is very similar to *P. maculata* in structure, behavior and regulation, but possesses some advantages over that species for experimental work. It attains a larger size, is more active, and can be obtained in unlimited numbers and all ages in this locality, while *P. maculata* is much less abundant. I found the same species in California some years ago (Child, '06), but was unaware at that time that it had been described.

In the present paper only certain experiments concerning the effect of anæsthetics on form regulation will be considered. It is possible by the use of dilute solutions of anæsthetics to control, modify and inhibit various regulatory processes almost at pleasure. For example, head-formation can be made a process of redifferentiation instead of regeneration in almost any desired degree (Figs. 14 and 16) or can be completely inhibited, according to the conditions of the experiments, and the same is true concerning the formation of a new posterior end and a new pharynx, and the regulatory changes in the intestinal branches. Moreover, the use of anæsthetics permits, in greater or less degree, an analysis of some of the various factors concerned, and finally, it is possible by this means to produce individuals capable of continued existence if returned to water which possess characteristics, or perhaps more properly, combinations of characteristics which do not occur in nature.

The anæsthetics chiefly employed in my experiments thus far are

alcohol, ether and acetone-chloroform, commercially known as chloretone. The effects of all are essentially similar in kind, but of course differ quantitatively according to the substance and the concentration. Ether, for example, in a concentration of 0.4–0.5 per cent. produces about the same effect as alcohol in a concentration of 1.5–1.6 per cent. In solutions of these concentrations, individuals and pieces have been kept alive as long as three months, though the resistance differs greatly according to the condition of the animals and various other factors, most of which can be controlled experimentally either directly or indirectly.

In order to avoid as far as possible decrease in concentration of the solution by evaporation the following method has been used. The animals or pieces are placed in Stender dishes of several hundred c.c. capacity with ground edges and a cover with ground groove exactly fitting the edge. The groove is filled with solution of the same concentration as that in the dish so that the dish is sealed so long as the fluid does not evaporate from the groove. After the dishes are thus closed they are placed in larger jars containing a liter or more of the same solution and these are sealed with vaseline and the covers weighted so that no escape of vapor or entrance of air is possible. And finally, all solutions are renewed every forty-eight hours and are made up immediately before using. In this way it is possible to compare the effect of the anæsthetic upon pieces of different size and from different regions of the body and also upon worms in different physiological condition.

This method makes possible the control and modification of form regulation in this species to a greater extent than any other which has been described. At present, however, only certain points will be considered, a full account of the experiments being postponed to a future time.

In several of my "Studies on Regulation" I discussed the changes in shape and proportion which occur in isolated pieces of various species of turbellaria and which, under the usual conditions, constitute an approach to the shape and proportions of the whole animal. These experiments with anæsthetics furnish new data which confirm my earlier conclusions, and it is some of these data which are to be presented here.

I. Experimental Data.

When whole individuals or pieces are placed in 1.5 per cent. alcohol or in 0.4–0.5 per cent. ether they lose the power of coördinated movement almost entirely for a time. After four to five days, however, they become in some degree acclimated to the new conditions and begin to move about very slowly, but with increasing vigor as time goes on, though they never attain the normal motor activity. Pieces including the old head begin to move about earlier than pieces without a head, for the latter must form a new head before they can regain the usual degree of motor activity, and regulation is greatly delayed in the solution. The important point for the present purpose is that for some days movement, and particularly coördinated locomotion, is almost completely eliminated. It is of interest to determine to what extent regulation occurs under these conditions.

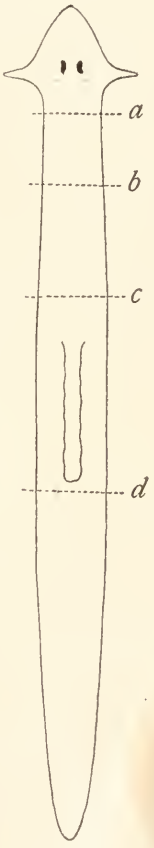
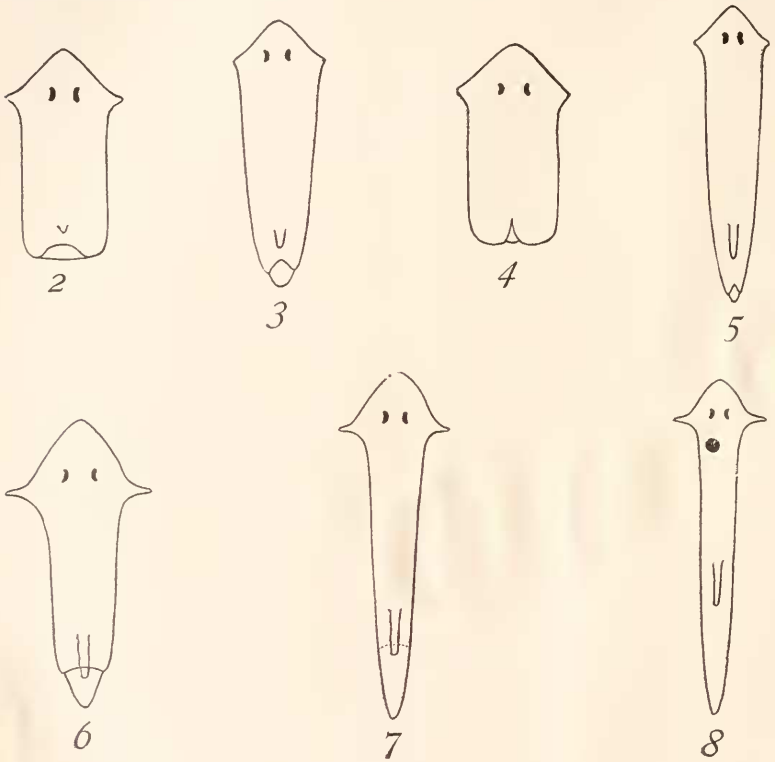


Fig. 1.

The first experiment to be described concerns pieces including that part of the body anterior to the line *b* in Fig. 1, *i. e.*, short pieces with the old heads. In Fig. 2 a piece of this kind after ten days in 1.5 per cent. alcohol is shown: during this time the piece has moved about but little and that chiefly during the last few days. Fig. 6 represents a similar piece after five days in water, Fig. 7 the same piece after ten days. Comparison of Figs. 2 and 7 shows that regulation in the alcohol is greatly delayed: a little new tissue has been formed at the posterior end, but, as a microscopic examination under pressure shows, it is still a mass of cells without any marked visible differentiation, and it can readily be seen that it is not used as a tail and is not attached to the substratum as the animal creeps slowly about; a small group of cells is present in the pharyngeal region, but these likewise show no marked differentiation. In water, on the other hand, a new tail has been formed which functions in the normal manner, contracting, extending and attaching to the substratum as the animal

creeps; the pharynx is well-developed and sections show that it possesses essentially the same structure as the pharynx in uninjured animals.

But the difference between the two pieces is most marked as regards change of shape. The piece in alcohol has not elongated at all, in fact it has decreased in length and it may be noted incidentally that the "auricles" on the sides of the head are greatly reduced. The piece in water (Fig. 7) has in the same



FIGS. 2–8.

length of time elongated to nearly twice its original length, has become much more slender and tapers posteriorly. This piece has moved about during regulation to an even greater extent than the uninjured animal, for short pieces with the old heads are usually more active than whole individuals.

After ten days the piece in alcohol gradually becomes more

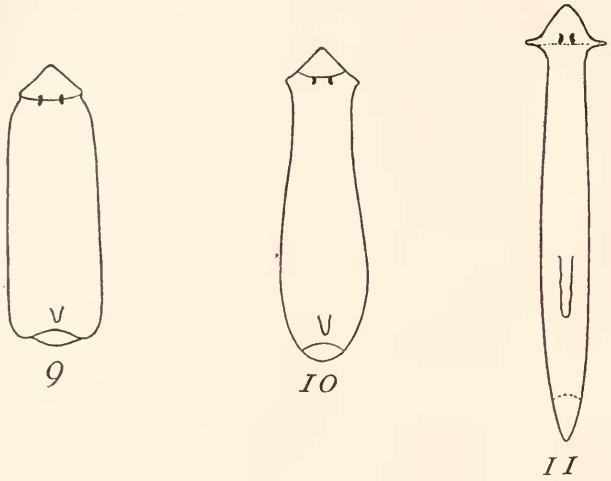
active, though it never attains anything like normal activity. At the end of twenty or twenty-five days it has acquired a shape like Fig. 3. The posterior end now functions as a tail to some extent and attaches itself to the substratum as the animal advances but the amount of new tissue has not increased. The piece may live for six weeks or more in alcohol but it never undergoes any appreciable further change in shape. The newly formed parts undergo some degree of differentiation but never attain the characteristic adult structure. Apparently the piece has attained approximately a condition of equilibrium for the conditions under which it is living.

If the concentration of the solution is gradually increased after the first three or four days it is possible to inhibit practically all regulation beyond the closure of the wound: the pharynx does not appear, no further growth of new tissue at the posterior end occurs, and the piece undergoes no elongation (Fig. 4). Under these conditions the piece does not acquire the ability to move about.

If now these pieces which have attained equilibrium in alcohol be returned to water they gradually resume the process of regulation, but with certain differences from pieces which have not been in alcohol. The chief difference for present purposes is that the outgrowth of new tissue at the posterior end does not proceed until it reaches the usual amount. The tail is formed almost entirely by a redifferentiation of the old tissue (Fig. 5). The pieces may undergo change of shape after their return to water until they attain practically the same shape as pieces which have not been in alcohol. Fig. 5 shows a later stage of Fig. 4 after its return to water and Fig. 8 will serve as regards shape for the late stages of either water or alcohol-water pieces.

These results, which are merely illustrations of what I have observed in several hundred pieces, permit certain conclusions of interest. In the first place it is possible to inhibit entirely the change in shape without inhibiting entirely the processes of redifferentiation and regeneration, and the change in shape can be stopped at any point without stopping entirely other regulatory processes (Fig. 3). On the other hand, if the growth of new tissue from the cut surface is inhibited in earlier stages (Figs. 2

and 4) the change in shape may occur in later stages (especially after return to water) without the formation of more new tissue (Figs. 3 and 5). It follows that the factors determining the change in shape must be in greater or less degree different from those determining the localization and growth of new parts. Moreover, the change in shape occurs only when the piece is capable of locomotion and it is in general proportional to the locomotor ability of the piece.

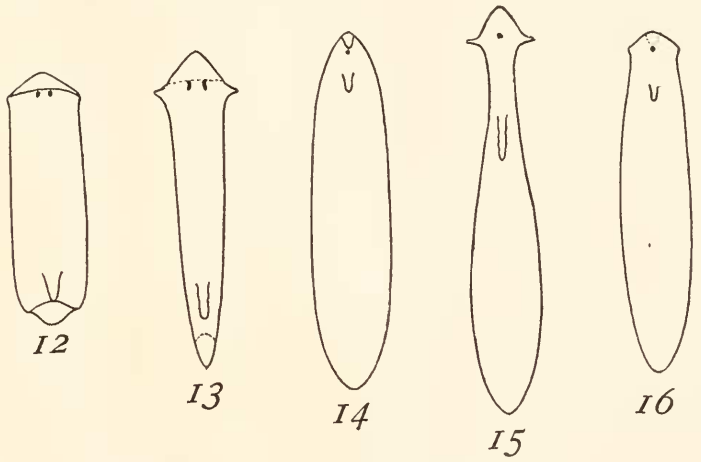


FIGS. 9–11.

But pieces which do not possess the old head afford even more positive evidence for these conclusions. Fig. 9 represents a piece corresponding to the region between the lines *a* and *c* in Fig. 1 after fifteen days in 1.5 per cent. alcohol. A new head has appeared, a small new pharynx is present as a mass of undifferentiated cells, and some new tissue has formed at the posterior end, but the piece as a whole shows no approach to the normal shape : it has undergone no marked changes in proportion. Incidentally it may be noted that the pharynx in such pieces appears much further posteriorly than in similar pieces in water. After seven days more the piece has the shape shown in Fig. 10. It moves about slowly, but its movements are different in character from those of pieces possessing the old head : here the posterior half of the body is very evidently not under complete control,

i. e., is not fully coördinated with the anterior region, and when the animal advances it is simply dragged along as a mass of inert material, its posterior end being only very rarely attached to the substratum. The shape of the piece suggests that the anterior part is being stretched by the strain upon it of the posterior portion, and I believe that is exactly what is occurring.

Similar pieces in water attain in the same length of time the shape and structure shown in Fig. 11. Pieces in alcohol of 1.5 per cent. do not change in shape much beyond the condition shown in Fig. 10, but if they are returned to water they regain their normal locomotor activity and may finally reach a shape like that of Fig. 11.



FIGS. 12–16.

In these cases a new head, a small new pharynx and some new tissue at the posterior end have appeared without any marked change of shape in the piece as a whole (Fig. 9). Evidently the change of shape and the localized formation of new tissue are not necessarily correlated.

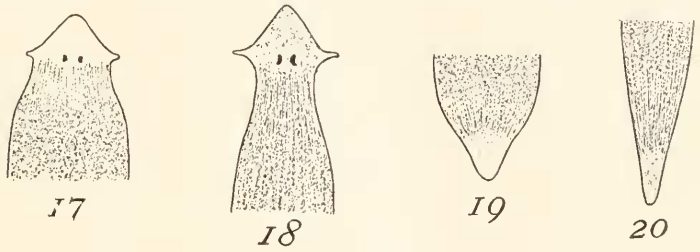
The same thing appears in Fig. 12, which shows a piece from the same region (*a* to *c*, Fig. 1) after fifteen days in 0.5 per cent. ether. Head, pharynx and posterior end have formed but no change in shape has occurred. This piece was returned to water at this stage and after seven days more had attained the shape shown in Fig. 13.

In another series pieces comprising the whole post-pharyngeal region were used (posterior to *d*, Fig. 1). These pieces, after eighteen days in 0.5 per cent. ether, had attained the condition shown in Fig. 14. A small head is forming, almost entirely by redifferentiation, at the anterior end, with one median eye, and a small pharynx is present. At this time half of the pieces were returned to water and half remained in ether. After nine days more the pieces in water had acquired the condition shown in Fig. 15 while those in ether were like Fig. 16. In the pieces returned to water the anterior half is greatly elongated but the posterior half remains much as before. In these pieces, as in the one described above, the posterior part was dragged about by the more active anterior portion. Gradually complete coördination returned and the posterior end began to attach itself to the substratum as the animal advanced and after this the shape gradually approached that of the normal animal.

Further data along this line could be presented but these cases are sufficient to show that it is possible to delay or inhibit the change in shape to any desired degree, and to induce its occurrence at any time. Moreover, the manner in which it occurs can be controlled and altered indirectly by using pieces of different sizes and from different regions of the body.

To my mind the evidence indicates very clearly that the change in shape is primarily a mechanical deformation of the body in consequence of the altered direction of the strains to which it is subjected as the animal advances. To control the change experimentally we have only to control the locomotor activity. In several of my earlier papers (Child, '02, '03, '04*a*) I have described the method of locomotion in certain species of turbellaria: in *Planaria* longitudinal strain arises in essentially the same manner as in the other forms discussed, the use of the posterior region as an organ of attachment being one of the chief factors. Moreover, there is considerable direct evidence that the tissues of a region undergoing change of shape are being stretched. In regions where the decrease in width and the elongation of the old parts begin, the chromatophores are always greatly elongated, and their elongation is greatest where the change in shape is most rapid. As the change goes on they become drawn out into long

lines. Figs. 17 and 18 indicate this change in shape of the chromatophores in the region posterior to a new head, and Figs. 19 and 20 for a region anterior to a new tail. In the pieces in alcohol and ether this change in shape of the chromatophores appears only when the change in shape occurs, not when the new tissue is formed. In cases where the change of shape is inhibited in the anæsthetic (Figs. 2, 4, 9, 12) it does not appear at all, but if such pieces are returned to water, and the change of shape occurs, the stretching of the chromatophores also appears. In Fig. 14, for example, it did not appear so long as the piece was kept in the ether, but after several days in water it was most conspicuous in the slender anterior region (Fig. 15), this region appearing almost



FIGS. 17–20.

as if finely striped in the longitudinal direction. This change in shape of the chromatophores is actually a stretching, not a migration, for it is possible to select some particular spot which happens to be conspicuous for some reason and to observe its change of shape from day to day: in such cases it can be seen clearly that merely elongation not migration occurs.

A similar elongation is visible in the parenchyme cells in section. Stevens ('07) has recently described this elongation or orientation of the parenchyme cells and regards it as indicating migration, but Steinmann ('08) does not agree with her. As a matter of fact the specimens in which the change of shape is inhibited by anæsthetics show nothing of the sort even in regions adjoining those where new tissue is being formed, but if such pieces be returned to water the cells of the parenchyme become very distinctly elongated or oriented in the direction in which elongation of the body is occurring, even though no new tissue is being formed at the time. In short the change in shape or

arrangement of the parenchyme cells probably has nothing to do with active migration, but merely indicates the direction of the strain which produces deformation.

These histological features then, support and confirm the other observations, and all appear to show that the change in shape is primarily a deformation in consequence of strain rather than a complex physiological process.

There can be no doubt, however, as I have repeatedly pointed out that reactions of various kinds result from the strain and deformation: muscles and other tissues undoubtedly "adapt" themselves to the new relations of parts. In fact there is no apparent reason why the change of shape should not continue indefinitely, or at least until the elasticity of the tissues became involved, if nothing but the mechanical deformation took place. Undoubtedly "functional adaptation" to the altered strains occurs and this determines how far the change shall proceed. Sooner or later the tissues adjust themselves fully to the new conditions, *i. e.*, a condition of equilibrium is attained and change in shape ceases. Under the usual conditions this gives what we commonly call the "normal" shape, but I have shown above that under other conditions the shape may be different. No one would deny, I suppose, that the shape of cells is determined in many cases (*e. g.*, the polyhedral shape of blastomeres in many eggs, the polygonal outline of many epithelial cells, etc.) to a greater or less extent by purely mechanical factors. If this is the case it seems scarcely possible to deny that purely mechanical factors may be concerned in determining the shape and arrangement of parts in animals without hard structures and with tissues of a high degree of physical plasticity. That they are the only factors in such cases, or that because they are factors in some cases, they must be in others, I certainly do not and never have maintained.

In *Planaria* the width of the head is apparently an important factor in determining the width of the body behind it. The head itself is not involved to any appreciable extent in the change of shape. It furnishes, so to speak, a fixed point, or rather region at one end, and between this and the posterior end the change of shape occurs. Consequently, in short pieces, where the new head is small, the change of shape is very much greater than in

long pieces, where it is much broader. In short pieces possessing the old head and consequently capable of rapid and frequent locomotion, the change occurs much more rapidly than in other pieces and the body often becomes much more slender proportionally than that of the "normal" animal and tapers more toward the posterior end. In pieces from the posterior region of the animal the chief region of attachment forms the largest part of the piece. Since attachment occurs by the lateral margins as well as by the tip of this region, the change of shape occurs most rapidly in the regions just behind the new head, for these are the regions where the greatest change in the direction of the strains occurs.

Summing up the data presented, the first point of importance is that no necessary connection exists between the change of shape and the redifferentiation or regeneration of parts: the two processes can be separated from each other almost completely in time, and are often separated in space. Secondly, it is possible to control the change of shape, to inhibit it, to stop it at any point, or to allow it to proceed, by controlling the locomotor activity. And finally, the tissues in regions undergoing change of shape show very distinct indications of physical deformation in the direction of the strains. The conclusion which seems to me to accord most closely with the facts as they now stand is that the change of shape is primarily a mechanical deformation resulting, at least in large part, from the strains which arise in locomotion, these strains being altered in direction and amount in pieces as compared with the whole animal. The final shape, *i. e.*, the cessation of change is probably determined by the physiological reaction of the differentiating or redifferentiating tissues to the altered conditions, and the consequent establishment of an equilibrium.

II. Function, Form and Regulation.

Since the appearance of my earlier papers on regulation certain reviews and criticisms of the work have appeared. From some of these, particularly from Driesch's review (Driesch, '05) I can only conclude either that I have failed to state my position clearly, or that the reviewers have not become sufficiently acquainted with my work to understand what that position is. Driesch, for example,

has imputed to me certain views which I have not only never held, but which I have expressly repudiated more than once. And recently, in a brief reference to my work, Morgan ('07, pp. 373–4) has cited certain conclusions as mine, which are very different from those which I have reached. I have considered Driesch's criticisms elsewhere (Child, '07) and shall refer to these and other criticisms only incidentally. But a brief statement of my position with regard to certain features of the problems of form and regulation and with especial reference to mechanical factors, movement and use of parts and function in general seems desirable in connection with the new facts presented above.

1. *The Relation between Function and Form.*[1]

In a fully developed organ certain processes occur which are concerned with the maintenance of the organism as a whole. These processes may affect either the relations of parts to each other or the relations of the organism as a whole to the external world, or both. They are commonly designated as functions, *i. e.*, the adult organism may be regarded as a complex machine, which works or functions in a characteristic manner.

But the functions characteristic of the adult organs do not appear in development until a certain stage is reached and the organ possesses a certain structure. Apparently then, development up to the stage where function in the above sense begins is a process of machine-building. Roux's distinction between a formative and a functional stage in development expresses this idea.

But how is the machine constructed and what is the agency which constructs it? The material of which the machine is to be made must be acted upon, arranged, transformed, localized, differentiated, etc. Evidently this point of view necessitates the assumption of a special formative agent or agents of some kind. How shall we conceive this formative agent? Pangenes, determinants, formative substances, entelechies are some of the answers which have been given to this question. All these answers are much alike in that they regard the construction of the organism as a process analogous in some sense to the construction of a

[1] Cf. Child, '06*a*, p. 402, '07.

machine by human agency. When the machine is constructed it begins to function.

This point of view seems to me to be essentially naive and anthropomorphic : moreover it is responsible for certain "Scheinprobleme" which have arisen from time to time, and for certain barren lines of research, particularly in zoölogy, which has been dominated by hypotheses of this general character to a much greater extent than botany.

To my mind life is inconceivable without some sort of function in some sort of structure ; a living structure functions as long as it is alive ; its function is its life. If this view is correct, the organism is functioning in some manner at all stages of its existence, from the earliest to the latest. Function in this sense is physiological activity of all kinds, all transformation and transference of energy.

It is a well established fact that the special functional activity of organs is very generally a factor in their development and differentiation beyond a certain stage, and in the maintenance of their characteristic form and structure after development is completed. In the absence of this functional activity the organ does not develop beyond a certain point and does not persist indefinitely. Functional hypertrophy, atrophy from disuse, functional adaptation, etc., are terms used to designate these relations between function and form.

But the real difference between earlier and later stages of development consists, it seems to me, not in the absence of function at one stage and its presence at another, but rather in the difference in character of function in the different stages. If a particular kind of function determines the structure and differentiation at one stage, and we know that it does this, is there not a logical basis for the belief that the functions which exist in other stages are also formative factors in those stages ?

In other words, is there not good reason to believe that every stage of development is directly or indirectly the necessary consequence of the functional activity in the preceding stage ? According to this view each stage of development is a machine in the broad sense and each is the product of the activity of a preexisting machine. External factors play a part, particularly in

plants and in the simpler animals, in determining the character of the machine and its activity, but this fact does not essentially alter the case.

Viewed from this standpoint, development, from its earliest stages on, is just as strictly a functional process as functional differentiation or functional hypertrophy in the stricter sense. This view does not necessitate the assumption of any special formative factors different in character from functional processes as the factors behind or underlying development. The formative factors of each stage are the functional activities of the immediately preceding stage (plus external factors). The process of development of the organism is not essentially different from the process of maintenance after development. Indeed strictly speaking, development ceases only when death occurs. The germ cell is an organism possessing a certain structure and function, and this forms the starting point. The functional activity in this structure determines the next stage of development, *i. e.*, a change in structure and therefore a change in function. This process continues and at the same time gradually approaches a condition of physiological equilibrium.

This view of development is of course no more an "explanation" than is the assumption of an entelechy or that of determinants. As a point of view, however, and as a basis for attacking the problem of morphogenesis it possesses a certain value in that it does away with various assumptions and places the problem of morphogenesis on a strictly physiological basis. To say that all organic form and structure are functional in their origin is merely to say that the problem of morphogenesis is a physiological problem and nothing more.

This is the position which I have held since the beginning of my work on regulation. I have used the word "function" with reference to any and all physiological processes and activities at any and all stages of development and have repeatedly called attention to this fact.[1]

[1] It is somewhat surprising, therefore, to find Driesch ('05, p. 790; '07, p. 180) pointing out that I have put the cart before the horse in regarding function as a determining factor in form regulation, since, as he says, an organ develops "for function" and does not function until it is developed, or at least until a certain stage of development has been reached. The difference between Driesch and myself on this

When we come to consider the process of development somewhat more in detail we find that two complex groups of internal factors must be considered, viz., constitution and correlation. This, of course, is true of any machine: its function depends upon the constitution and correlations of its parts, provided, of course, that the necessary external conditions for function are present. In different organisms and in different features of development the two factors may of course play a very different part.

2. *Functional Regulation and Form Regulation.*

It follows from what has been said that all form regulation is, according to my definition, either directly or indirectly functional regulation, *i. e.*, the physiological processess in the structure involved determine what the result of regulation shall be (Child, '06*c*).

In certain cases among the turbellaria I have been able to control, inhibit and modify the process of form regulation to a greater or less extent by controlling and modifying, in most cases indirectly, the movement and use of the parts most intimately involved in the regulatory processes (Child, '02, '03, '04*a–c*, '05*a–d*). This work showed very clearly that movement and use of parts were important factors in certain cases and certain features of regulation: they may even be primary factors in determining certain results. It is just as certain, however, that in many other cases they play no part at all.[1]

point is in reality merely one of definition; he limits the term "function" to the processes of which I spoke in the first paragraphs of this section, while I regard all processes in the organism as functions. His criticism of my position is of course entirely beside the point since he substitutes his definition for mine. Physiological processes of some kind certainly occur even in the earliest stages of development, and that these are functions of an existing structure cannot be doubted. I believe that they are also the formative factors in development.

Morgan ('07) calls attention to the fact that the functional idea is not new. This of course is true; it is the position which every physiologist must hold in one form or another. I have never considered that I was formulating a new hypothesis of development; I have merely attempted to apply certain physiological ideas to the phenomena of form regulation. Among botanists this view has been very generally held for a long time.

[1] Morgan ('07, p. 374) apparently believes that I regard movement as a universal factor in form regulation, for he calls attention to the fact that movement does not occur in many cases of regeneration. But I have repeatedly asserted that my conclusions upon the effect of movement concerned only certain species and certain features of regulation (Child, '02, p. 218, p. 229; '04*a*, p. 131; '04*b*, p. 467, etc.).

As I pointed out concerning *Leptoplana* (Child, '04*a*) movement is undoubtedly not a factor of importance in determining the formation of new tissue at a cut surface, but experimental evidence indicates clearly enough that it is a factor in determining the rapidity, amount and direction of growth and to a greater or less extent the character of its differentiation.

In the experiments on *Planaria* described above, the head and pharynx, for example, do not attain their "normal" shape and structure when movement is largely inhibited by anæsthetics. By the use of the higher concentrations it is possible to inhibit in almost any desired degree the process of form regulation so far as visible morphogenesis is concerned. Pieces which in water form heads very rapidly may be prevented entirely from forming heads by the proper use of the anæsthetic, or almost any intermediate condition between these two extremes may be attained by the formation of incomplete or partial heads. Pieces incapable of forming heads in the anæsthetic, regain their original power when returned to water. But it does not in the least follow from these facts that head formation is entirely the result of actual movement. My position is, and has been, that movement is merely one of the functional activities concerned in development, and which usually concerns later stages to a greater extent than earlier. But in regeneration in the turbellaria and in various, though by no means all, other forms a peculiar condition exists in that new tissue adjoins fully developed parts which may be in active movement. There can be no doubt that movement of these adjoining old parts influences conditions in the new tissue and so affects the result, either quantitatively or qualitatively or both. But movement and functional activity in the stricter sense, in which Driesch uses the word, are certainly far from being universal factors in either form regulation or ontogeny.

Whatever the importance of movement in a particular case, it is merely one of a great variety of functional factors. In many cases movement and motor use of parts are not concerned at all in regulation and in some other cases it is evident that while movement may affect the later stages this movement is possibly only in consequence of preceding regulation. In *Cestoplana* (Child, '05*b*), for example, where the posterior portion of an an-

terior piece becomes visibly "posterior" as regards the character of its movements before any marked structural changes occur, regulation must have occurred before such changes in movement could appear.[1]

3. *Mechanical Regulation and Morphallaxis.*[2]

In the first of the "Studies" the idea of mechanical regulation as the chief factor in the change of shape in pieces of *Stenostoma* was developed. According to my conception mechanical regulation is primarily a mechanical deformation of physically plastic tissues. Whether we consider regulation as a return or approach to the normal condition after a disturbance of this condition (Driesch, '01), or as a return or approach to a condition of physiological equilibrium after a preceding condition of equilibrium has been disturbed (my own definition), mechanical regulation in my sense

[1] In my paper I referred to these changes as functional regulation and called attention to the altered character of the movement as indicating that they had occurred, but while the movement is undoubtedly a factor in what follows, it would be absurd to suppose that it is the primary factor in such a case.

[2] Some years ago, in describing the course of regulation in *Planaria maculata*, Morgan called attention to the peculiar changes in shape which the pieces undergo, these changes consisting mainly in a decrease in width and an increase in length. Concerning this process he says: "Thus the relative proportions of the planarian are attained by a remodelling of the old tissue. I would suggest that this process of transformation be called a process of morphallaxis" (Morgan, '98, p. 385). Later ('00) he applied the same term to the changes in shape and proportion in *Hydra* and other forms. So far as I am aware, Morgan has not stated at any time whether the term "morphallaxis" is to be applied to the whole process of form regulation in *Planaria* and other forms, including the regeneration and redifferentiation which occurs, or only to the changes in shape and proportions of the piece. From the quotation given above it would appear that he intended it to apply only to the change in shape and proportions, but in his latest statement ('07, p. 15) he apparently applies the term to the whole process of form regulation by redifferentiation. In my earlier "Studies" I used the term with reference only to the changes in proportion and shape: later, however, I substituted "change in proportions" for it as less ambiguous for my purposes (Child, '05*a*, p. 253). Driesch ('01) considered the term as synonymous with "Restitution durch Umdifferenzierung," a very different meaning from that which I had given it in my work. It is probable that Driesch's misunderstanding of my conclusions concerning form regulation is in part due to our different interpretations of this term.

In several of my earlier papers the word "form" was used for "shape" and "outline." I agree with Driesch that this use of the word may be misleading, but I was careful to distinguish between form in this sense and structure, and pointed out that the factors concerned with change in form probably had nothing to do in many cases with change in structure (Child, '02, p. 218).

is theoretically possible, provided certain characteristic mechanical conditions exist in the normal animal and are altered in characteristic manner in the piece. In several of the "Studies" the changes in shape in pieces of *Stenostoma*, *Leptoplana* and *Cestoplana* were shown to be apparently primarily mechanical regulations (Child, 02, '04*a*, '05*a*, '05*c*) and it was suggested that similar processes might occur elsewhere. The recognition of mechanical regulation is not in any sense an attempt to interpret regulation in general on a mechanical basis (cf. Child, '02, p. 229): it concerns merely changes in shape and outline. Mechanical regulations are possible only under the conditions mentioned above, and whether they occur or not can only be determined by investigation of each particular case. It is not necessary to suppose that all cases of "morphallaxis" (in the sense of change in shape and proportions) are mechanical regulations. If they are they certainly depend on different mechanical conditions in different cases. In *Hydra*, for example, the factors determining the change in shape are certainly not the same as in *Planaria*, and may not be mechanical at all. In the cases which I have considered mechanical strains arise in connection with locomotion and these strains are altered in a characteristic manner in isolated pieces and, as I have shown, the changes in shape are exactly what might be expected in physically plastic material under these conditions. Mechanical regulation is to be expected only in organisms or parts possessing a considerable degree of physical plasticity and where skeletal structures are not concerned. As regards the shape of animals in general gross mechanical factors are certainly unimportant as compared with others, or not concerned at all, and I have never even suggested that such factors were of general significance (cf. Driesch, '05, p. 790). On the other hand, it seems to me absurd to deny that characteristic strains or pressures existing in plastic material may play some part in determining shape.

That the changes in shape in pieces of *Stenostoma*, *Leptoplana*, *Cestoplana*, and *Planaria* are not primarily "functional adaptations" (cf. Driesch, '05, p. 766) is, I think, evident. A functional adaptation, as I understand it, is a change in structure which involves a change in functional capacity, either an increase

or a decrease or a modification in kind according to the character of the factor inducing the change. The change in shape in the pieces of *Planaria*, etc., does not in itself involve any such change in functional capacity, moreover, functional adaptations to mechanical tension and pressure consist, usually if not always, in an altered resistance to the mechanical factor, not in a change of shape which accords with the laws of mechanics. The change in shape in these turbellaria is essentially similar to what would occur under the same conditions in any material of similar physical constitution and there is not the slightest evidence that it results from a change in functional capacity.

There is little doubt, however, that functional adaptations result from the change of shape: as was suggested above, it is probable that the cessation of the change and the final attainment of a more or less characteristic shape, *i. e.*, a condition of equilibrium, is in part the consequence of the functional reaction of the differentiating and redifferentiating tissues to the altered mechanical conditions. In other words, the change of shape brings about the functional adaptation instead of resulting from it.

But even though these changes in shape do not appear to be functional adaptations in Driesch's sense, they are functional regulations in my sense, and probably the simplest possible kind of functional regulation.

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March, 1909.

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SOME ABNORMALITIES AND REGENERATION OF PLEIOPODS IN CAMBARUS AND OTHER DECAPODA.[1]

CHARLES ALBERT SHULL.

Introduction.

The two crayfishes whose abnormal appendages are described in this paper were found among a small number of specimens of *Cambarus virilis* Hagen, which were captured near the city of Chicago in the autumn of 1904. The discovery of these abnormal pleiopods led to an experimental study of the regeneration of the abdominal appendages in the crayfish, the results of which are given herein. The earlier experiments were unsuccessful owing to imperfect methods of handling the material, and to the use o mature animals in which the moults were infrequent. The later experiments, with improved methods of caring for the material, and with small immature specimens, have yielded satisfactory results.

These experiments were begun in the Hull Zoölogical Laboratory of the University of Chicago, continued in the Biological Laboratory of Transylvania University, and completed in the Marine Biological Laboratory of the Brooklyn Institute of Arts and Sciences at Cold Spring Harbor, Long Island. I wish to express my thanks to Dr. Davenport, director of the Marine Laboratory, who kindly placed the facilities necessary for the completion of the work at my disposal; also to Dr. A. E. Ortmann, of the Carnegie Museum, Pittsburgh, to whom I am indebted for identification of the species used in the experimental work; and to Dr. Faxon, of Harvard, and to Dr. Hans Przibram, of Vienna, for examining the abnormal specimens.

Historical Summary.

The regeneration of the abdominal appendages of decapod crustaceans has been a subject of investigation for many years, but

[1] Contributions from the Biological Laboratory of Transylvania University, No. 2.

not until recently has a critical study of it been attempted. A review of the earlier literature relating to regeneration in the Decapoda has been given by Miss Steele, '04; it is necessary, therefore, to mention only a few of the later papers upon whose results my own observations have some bearing.

Morgan, '98, desiring to test Weismann's hypothesis, that regeneration is an adaptation, experimented with *Eupagurus longicarpus* to determine what relation, if any, existed between the power of regeneration and liability to injury. Because the hermit crab lives in shells where the abdominal appendages are protected from injury, whereas the thoracic appendages are not so protected, these two series of organs were chosen for this experiment. He came to the general conclusion that no such relation existed, and further supported this view by a second paper in 1900. The latter paper was based upon experiments in which the thoracic appendages were removed at unusual levels.

He found in the course of his experiments that the abdominal appendages of *Eupagurus* did not regenerate readily, although slight regenerative power existed. He suggested that this rarity of regeneration "may be connected in some way with the amount of food supply brought to the region from which they arise."

Miss Steele, '04, experimenting with *Cambarus virilis* and *C. gracilis* has obtained results similar to Morgan's. She says, speaking of the swimmerets: "I have found none to regenerate except the first pair in the male. . . . In the case of the other abdominal appendages except the sixth pair, regeneration, if it does take place, is very slow in beginning."

Emmel, '04, reported the observation of regeneration in the first appendages of the lobster. He experimented with the other appendages and says: "In experiments with the other four pairs of abdominal appendages or swimmerets, positive results were obtained in the second and third pairs, and it seems safe to say that all the swimmerets will regenerate."

Haseman, '07, mentions the regeneration of swimmerets in *C. propinquus*, giving figures showing the progress of differentiation, but he does not mention the fact that the regeneration of these appendages was considered doubtful.

A discussion of the results obtained by Morgan and Miss Steele will be found in connection with the discussion of my own observations in a later portion of this paper.

Abnormal Swimmerets.

Anomalous variations not of congenital origin are of little interest to the student of evolution; for it has never been definitely demonstrated that any acquired somatic variation is inherited. Moreover, no very important laws of variation are likely to be discovered by a study of teratological specimens. Nevertheless, it is advisable to place interesting cases of abnormal growth and regeneration on record, for we may finally acquire a knowledge of the physiology and mechanics of development sufficient at least to explain the origin of such anomalous variations.

The swimmeret shown in Fig. 1 is the right appendage of the second abdominal somite of a female *C. virilis*. The pleuron is much deformed, as is readily seen if compared with the normal pleuron of the opposite side of the same somite. The coxopodite of the abnormal appendage is much larger than normal. From this enlarged basal piece arise two appendages, one of which, near the posterior side of the coxopod, seems on casual observation to be a beautiful case of duplicity. The parts are perfectly doubled from the proximal portion of the basipodite outward. The two pleiopods thus united are normal in every particular beyond the point of union, the posterior member being somewhat larger than the anterior. From the anterior portion of the large coxopodite, completely separated in point of origin from the double pleiopod, arises another appendage which is uniramous, but of a size and structure typical of the endopodite of the second pleiopod.

It is an interesting circumstance that the first pleiopod lying immediately anterior to the one just described is also abnormal. This swimmeret is shown in Fig. 2. The lower portion of the appendage is much enlarged, and at the proximal end of the basipodite, there is a posterior projection set with stiff hairs, reminding one somewhat of the structure of the coxopodite of the pereiopods.

The third abnormal swimmeret is from a male *C. virilis* which

was found to have three pairs of appendages instead of two, modified for sexual purposes. The right swimmeret of the pair is shown in Fig. 3, the left one being exactly like it. The character of the modification in this third pair is just the same as that of the second, except that the projection of the endopodite is smaller than on the second. The first and second pairs of appendages are perfectly normal. Moenkhaus, '03, has reported an exactly similar case in the same species, and so far as I know these are the only two cases on record.

The Problem, Materials, and Methods.

Being convinced that the abnormal swimmerets of the female *C. virilis* described herein were the result of regeneration after a somewhat extensive injury, and having before me the work of Morgan on *Eupagurus longicarpus*, and of Miss Steele on *C. virilis* and *C. gracilis*, I determined to perform a series of experiments with the crayfish in order to ascertain whether or not the abdominal swimmerets regenerate, and if so, under what conditions. These experiments were begun in 1905; and after two years of rather unsuccessful work the choice of material and the methods of handling it were so improved that gratifying results have been obtained.

At first attempts were made to keep the crayfishes in aquaria of running water. A large aquarium was divided by partitions of galvanized iron netting into a number of compartments, each about 30 cm. square. Each compartment was provided with gravel and flat stones, and fresh water from Lake Michigan was kept running constantly at a depth of about 5 cm.

The aquarium was cleaned frequently, and all precautions were taken to keep the animals in sanitary conditions; but in spite of the great care exercised, the crayfishes would die after several weeks of confinement. Since my first material was adult, and moulted therefore infrequently, death took place before any moults occurred. Many variations of these conditions were tried in an attempt to secure more favorable results, but without success.

During the last two years a method has been employed, which has obviated all difficulties, and has given entirely satisfactory

results. Young specimens of *Cambarus* (*Bartonius*) *bartoni* Fabr., probably at the beginning of their second and third years were taken on March 24, 1907, from a small stream which crosses the Bryan Station pike about three fourths of a mile northeast from the city limits of Lexington, Ky. The specimens used are not like the typical *C. bartoni* of the eastern United States, but have a narrower areola, less spiny carpus, and a shorter but broader rostrum than the eastern form. Dr. Ortmann states that they agree with specimens described by W. P. Hay in the 20th Ann. Report of the Ind. Geol. Survey, 1895, after comparing the living specimens with those in the Carnegie Museum from Mitchell, Lawrence Co., Ind.

Each specimen was put into a tumbler with water not quite sufficient in amount to cover it. A small piece of mica schist coming not quite to the surface of the water, was placed in each tumbler so that the crayfishes could crawl upon it and expose themselves to the air at will. The tumblers were kept nearly covered by glass plates to prevent accidental desiccation. Care was taken not to cover the dishes entirely, as the air was found to become overcharged with carbon dioxide in a short time if so covered. The water was changed completely three or four times per day, and even oftener on very hot days; for crayfishes seem unable to endure warm water for any great length of time.

The water used in these experiments was supplied by the city water works of Lexington. This water is filtered through large Jewell filters before being pumped into the water mains, and is exceptionally pure. The complete change of such water every few hours rendered the multiplication of bacteria or growth of other fungous plants rather difficult, and tended to prevent the accumulation of any sediment.

When it appeared that any fungous growth was forming upon the appendages and about the thoracic region, each crayfish was treated for a few minutes with a bath of copper sulphate. The vessels and stones kept in them were occasionally treated with the same solution. The copper sulphate was used with a strength of one part to a million, when the animals could be left in it for some time. Solutions much stronger than this, one part in ten thousand, can be used for a few minutes at a time with entire suc-

cess in destroying bacteria and fungi, and without injury to the crayfishes, provided they are washed thoroughly several times in plenty of pure water after the bath to cleanse their gills of the copper sulphate.

Young crayfishes are voracious creatures, and need to be fed frequently. I have fed them every day with entire success, but they soon tire of a uniform diet. Several kinds of food were used; and by rotation, so that they never received the same kind of food on successive days, their appetites were retained. Fresh raw beef was given them twice a week, and raw potatoes and pieces of *Myriophyllum*, which was kept growing in the laboratory, were used as vegetable foods. The *Myriophyllum* was covered with slime, which was found to contain unicellular algæ, rotifers, nematode worms, annelids, such as *Æolosoma hemprichii*, and other kinds of small animals. This slimy material was especially esteemed, and probably most nearly represents their normal diet. This food material was left in the glass with the animals for an hour or two, after which the remains were removed and fresh water placed in the vessels.

Using these methods, I have kept them alive for months in perfect health, with rapid growth during the moulting season. Occasionally a death would occur among them, but these fatalities were always obviously due to special causes, not to any general defects in the methods employed.

After ecdysis the cast off exoskeletons were used in the preparation of the drawings.

Experiments.

The experiments here described are a few typical ones selected from a series, all of which gave similar results. The numbers correspond to those used while recording notes on individual experiments.

No. 4. *C.* (*Bartonius*) *bartoni*, ♀. Fifth right abdominal appendage removed March 24, 1907. The appendage was cut off, leaving a short stump attached to the body (Fig. 4).

The first moult occurred in the afternoon of March 27, three days after the operation. No regeneration could be noticed, but the wound was perfectly healed (Fig. 5). The second moult

occurred during the night following May 6, 1907. Between March 27 and May 6 no change could be detected even by use of the microscope. But that changes were taking place beneath the chitinous cuticle is evident from the expansion of the regenerating limb, which occurred immediately after this second ecdysis (see Fig. 6).

A third moult took place on June 19, 1907, at which time the appendage again expanded, reaching the size and condition shown in Fig. 7. The regenerated appendage measured at this time 3.3 mm. in length; the one on the opposite side of the same somite 4.8 mm. (Fig. 8). The regeneration was equal to nearly 69 per cent. of the normal size from March 24 to June 19, a rate which I consider by no means slow.

Owing to an unfortunate accident this crayfish was killed on the morning of July 5, 1907. Had it lived until after its fourth moult it would probably have shown a much more nearly complete regeneration of the appendage.

No. 7. *C.* (*Bartonius*) *bartoni*, ♀. Fourth left pleiopod removed March 24. The first moult after the operation took place the night of April 22, 1907. The amount of regeneration while not extensive was plainly visible. The second moult occurred May 25, 1907. The regenerating appendage expanded to nearly half the natural size, immediately after the exoskeleton was cast (Fig. 9).

No. 3. The same as No. 7 except that the fourth pleiopod was removed from the right side instead of the left. The first moult, on March 30, showed a small whitish papilla a little over one mm. in length, projecting from the base of the amputated limb. This projection increased slightly with age, but this individual was killed in July by the water in the vessel receiving direct sun-light through a partially open window blind, thus becoming much overheated. Fig. 10 reveals internal development. A later moult would probably have given results similar to No. 7. This specimen was probably a year, or perhaps even two years, older than No. 7, and consequently only one moult was recorded between March 24 and July 5, while some of the smaller, younger specimens moulted three times in a shorter period (cf. No. 4).

No. 2. *C.* (*Bartonius*) *bartoni*, ♂. The third right appendage was cut off March 24. On May 9, no moult having occurred, the stump of the appendage was cut longitudinally by means of sterilized scissors. The moult occurred on June 17, but no abnormal growth was noted. The appendage was perfect, and showed regeneration of more than 50 per cent. in size (Fig. 11).

No. 5. *C.* (*Bartonius*) *bartoni*, ♀. The second left appendage was removed March 24, 1907. The first moult occurred on May 8, 1907, and the regeneration amounted to about 30 per cent. The second moult took place on June 26, 1907, when the appendage showed about 65 per cent. of complete regeneration. Fig. 12 shows the point of amputation, and Fig. 13 the appendage after the second moult.

No. 6. *C.* (*Bartonius*) *bartoni*, ♂. Sixth right appendage was amputated just beyond the basal joint March 27, 1907, as shown in Fig. 14. It moulted the following day. A very narrow edge of white tissue was visible along the cut edges, but it is not probable that any regeneration had taken place. During the time preceding the next moult which occurred on May 27, the basal portions of the rami increased somewhat in size (Fig. 15), and after moulting the appendage was about one half natural size, and perfectly formed (Fig. 16).

A series of experiments on the pleiopods of *Palæmonetes vulgaris* was carried on at Cold Spring Harbor, and regeneration of all the abdominal swimmerets takes place rapidly in young specimens.

At present a series of experiments to test the regeneration of the antennæ from various levels and to compare the rate of regeneration of pleiopods and pereiopods in *C.* (*Bartonius*) *bartoni* is being carried on; but the data are insufficient as yet to permit a general statement regarding either phase of the series.

Discussion.

A. *The Abnormalities:* — As far as I have been able to ascertain, only one abnormal abdominal appendage has been recorded for any of the decapod crustaceans. In as much as all abnormal pleiopods described herein or elsewhere have been discovered accidentally, the rarity of such records is due in part, perhaps, to

lack of careful observation; but the examinations which have been made show that they occur infrequently.

The abnormalities presented here are of interest for several reasons. The abnormal pleiopod is evidently the result of a regeneration after extensive injury in one instance, and just as evidently the result of undisturbed growth processes in the other instance. That is, the condition is congenital and may be considered as having arisen by mutation.

It would appear that the swimmerets shown in Figs. 1 and 2 probably resulted from an injury which removed part of the pleuron, and tore the first and second pleiopods near the base of each, as represented diagrammatically in Fig. 17. The coxopodite in Fig. 1 is abnormally large, which condition may possibly be explained by the fact that a large area was exposed when the mutilation occurred.

Very few genuine cases of duplicity have been described. Bateson mentions only four cases, all chelæ; Herrick has figured a double chela of a female lobster, and Zeleny has described a double chela of *Gelasimus pugilator* which regenerated instead of a normal single one during the course of his experiments.

Bateson states emphatically that "in arthropods and vertebrates such a phenomenon as the representation of one of the appendages by two identical appendages standing in succession is unknown. No right arm is ever succeeded on the same side of the body by another arm properly formed as right, and no crustacean has two right legs in succession where one should be."

While this supernumerary appendage may be regarded as a complementary image of the normal one, and therefore a left appendage instead of a right, there is nothing in the structure of either member to indicate that such a relation exists. The members are not imperfect, and are placed in succession, differing in these two respects from the cases admitted by Bateson.

At first appearance the coxopod of the abnormal first appendage is very much like that of the last pereiopod. And the anterior uniramous part of the second pleiopod is like the first pleiopod in being uniramous, but is a typical endopodite. There is a mere suggestion here of a shifting backward of the series of organs, a condition to which Bateson applied the term backward homœosis.

However if the injury actually took place as indicated in the diagram referred to above, the most plausible explanation which can be offered is in harmony with Bateson's statement. The regeneration should lead theoretically to triplication, that is, to the production of three appendages, two supernumerary ones on each cut appendage; and these on the right side of the body should stand as a left between two rights. The abnormal pleiopod shows defective regeneration only in the suppression of the exopodite of the anterior supernumerary appendage. The rounded projection of the first accessory pleiopod may be explained similarly as the fused basal portions of the two supernumeraries, whose tapering jointed ends have been completely suppressed.

The abnormalities have probably arisen after an injury caused by some force acting in the direction of the arrow in the diagram, Fig. 17, which produced two breaking surfaces, from which the new pleiopods arose, following the laws of symmetry for supernumerary appendages as stated by Bateson.

The abnormal appendage shown in Fig. 3, is a case of backward homœosis. It would be of interest to know the hereditary behavior of such unisexual characters if bred in confinement.

B. *Experiments.* — Few investigations of the regenerative power of the abdominal appendages of the Decapoda have been made, and the results obtained have not been entirely satisfactory. Morgan's experiments on *Eupagurus longicarpus* in 1898 showed that a slight power of regeneration existed in the appendages of two or three of the individuals he used; but his experiments extended over too brief a period of time to secure any marked regeneration. The first experiment was continued only twenty days, and the second for twenty-eight days. And the conditions under which the material was kept were possibly not the most sanitary, as the fact that over 40 per cent. of the individuals died during the twenty-eight days would seem to indicate. One would hardly expect regeneration to be rapid under conditions in which life itself could scarcely be maintained. My experience has shown that although regeneration may occur after an operation, and become visible without an intervening moult, it usually does not do so in the pleiopods. None of the individuals Morgan used was kept until a moult had occurred.

Miss Steele's experiments on *C. virilis* and *gracilis* were carried on for a sufficient length of time, but she found it difficult to provide the sanitary conditions necessary to such prolonged experimentation. The specimens used in her work were probably too old to give the best results. They measured not more than three inches in length, a size which would indicate that they were several years old at least. The younger the specimens used, the more frequently the moults take place. Those used in my experiments measured from one and six tenths to two and five tenths cm. in length, and were probably at the beginning of the second or third year. Two individuals measuring five cm. in length were also used. These did not yield as satisfactory results although some regeneration occurred. The difference was probably more in the frequency of moulting than in anything else. While one moult took place in these older ones, I could secure three in the younger. And to have kept the older individuals until the same number of moults occurred as in the younger ones, would have required two years instead of four or five months. The use of more nearly adult material, and the fact that slight attention was paid to the swimmerets may account for the slight regenerative power which Miss Steele was able to report.

My experiments show that the swimmerets of young specimens of *C.* (*Bartonius*) *bartoni* regenerate rapidly, and that the regeneration of any of the appendages may be practically completed in a single season of growth. The regeneration is not particularly slow in beginning, having become visible in one instance only six days after the operation was performed. It is usual, however, to find that the regeneration begins and takes place under the old exoskeleton, without showing any visible indications that the new parts are forming. For instance, No. 9 moulted on March 28, four days after the operation, and no regeneration could be noticed. The next moult took place April 30, but during that time no indication whatever that regeneration was occurring could be seen. Nevertheless, when the moult occurred it was evident that regeneration had taken place. If the experiment had been continued for only thirty days, the conclusion naturally drawn would have been that regeneration either did not occur, or was " very slow in begin-

ning." As a matter of fact there is no way to tell how soon after March 28 the regeneration did begin, if indeed it had not already begun at that time.

To explain the slight regenerative power which he found in the abdominal appendages of *Eupagurus*, Morgan suggested that the food supply of these organs might be considerably less than that of the thoracic legs. It seems to me quite unnecessary to make this assumption, especially since I have shown that there is a rapid and complete regeneration of the swimmerets in young specimens of *C.* (*Bartonius*) *bartoni*. In this connection Emmel, '04, observed that swimmerets in the lobster will regenerate more rapidly than the pereiopods if the latter are cut "only a relatively short distance below the breaking plane." And he questions whether the supply of food material can explain the comparative difference in the regeneration of pereiopods and swimmerets. Moreover, if the limited food supply is responsible for lack of regenerative power how must we regard the regeneration of two supernumerary appendages in the abnormal swimmeret figured? It seems to me that some other explanation must be offered for the difference in regenerative power.

It has been a common experience with those who experiment with regenerative tissues, that the regeneration is always more rapid and complete in young individuals than in old ones. This fact is due probably to the greater plasticity, the more active and mobile condition of young tissues. They are more nearly embryonic in character, differentiation is not so complete, nor so fixed as in the older tissues. Emmel's ('08) recent work on the reversal of asymmetry in the lobster lends emphasis to this statement. He says: "In the first four stages of the lobster's development, a crusher may be produced on either the right or the left side of the body by the autotomous amputation of the chela on the opposite side — the regenerated chela becoming a nipper. During the fifth stage, although the chelæ are still symmetrical, the possibility for such experimental control disappears."

Since differentiation is known to proceed at different rates in different parts of the body, we may suppose that one part retains its primitive condition longer than another. If any portion of the adult regenerates less rapidly than another portion, may

it not be an index of the comparative plasticity of the two parts? The more rapid regeneration of the thoracic appendages than of the swimmerets in the adult crayfish, may be due to a longer retention of the plastic embryonic condition of the cells in the region of the breaking joint by the former, certainly not to any proportionate difference in the food supply of the two series of organs.

The important point in Morgan's results which he properly emphasized, was that regeneration did take place, however slight it might be. While we cannot say that the abdominal appendages of crayfishes are never injured, yet injuries to them are rare in nature. Examination of hundreds of individuals has shown that there is a remarkable uniformity of size and structure in these organs, a condition which could not exist if mutilations were frequent. Yet these appendages possess as high a power of regeneration in youthful stages as any of the appendages which are so frequently torn away. My experiments strengthen very much the evidence in favor of Morgan's statement that there is no relation between power of regeneration and liability to injury.

Conclusions.

The following conclusions may be drawn from the study of abnormalities, and the results of the experiments described in this paper.

1. The abdominal swimmerets of *C.* (*Bartonius*) *bartoni* Fabr. all possess a high power of regeneration in immature specimens. Since regeneration of swimmerets has been noted in *Cambarus propinquus*, *Palæmonetes vulgarus*, *Homarus americanus* and *Eupagurus longicarpus*, it is probable that the decapods generally possess this power, especially in young individuals.

2. This regeneration usually cannot be seen till after one, and sometimes two, moults have occurred, due to masking of the regeneration by the exoskeleton.

3. Slow regenerative processes in the swimmerets of the older individuals are due probably to a lower degree of plasticity in the protoplasm rather than to insufficiency of the food supply.

4. Injuries may occur, but they are rare in swimmerets, and the power of regeneration and liability of the parts to injury are apparently independent.

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Explanation of Plate I.

All figures except Fig. 17 are $\times 5$.

Fig. 1. Second right pleiopod of *C. virilis* Hagen, ♀, showing two supernumerary appendages.

Fig. 2. First right pleiopod of the same specimen.

Fig. 3. Third right pleiopod of *C. virilis*, ♂, modified for sexual purposes.

Figs. 4–7. Successive steps in the regeneration of the fifth right pleiopod in *C.* (*Bartonius*) *bartoni*, ♀.

Fig. 8. Left pleiopod from same somite as Fig. 7, without regeneration, drawn to same scale.

Fig. 9. Fourth left pleiopod of young *C.* (*Bartonius*) *bartoni*, ♀, after two moults.

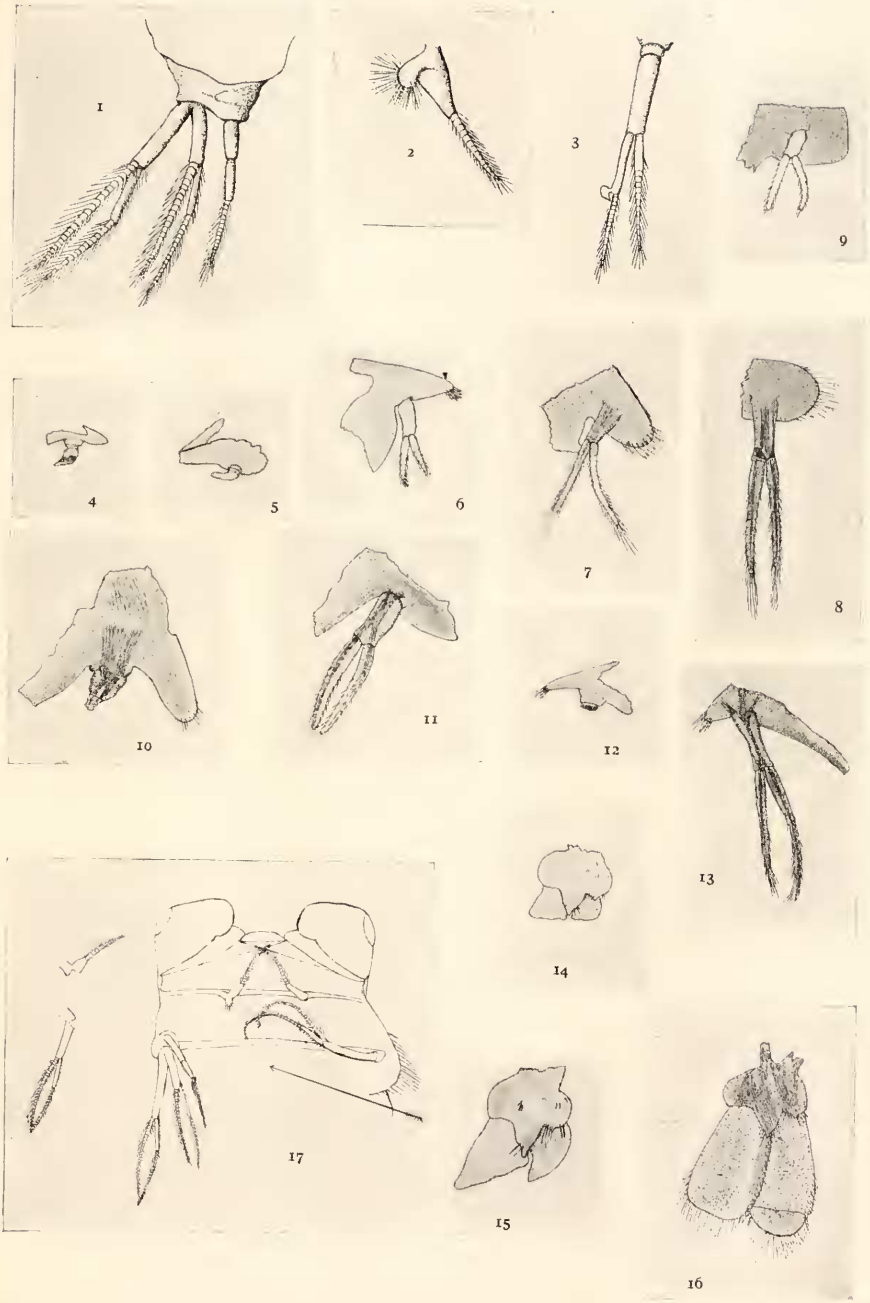
Fig. 10. Fourth left pleiopod of older *C.* (*Bartonius*) *bartoni*, ♀, after three and one half months.

Fig. 11. Third right pleiopod of *C.* (*Bartonius*) *bartoni*, ♂, after one moult.

Figs. 12–13. Second left pleiopod of *C.* (*Bartonius*) *bartoni*, ♀, condition immediately after amputation, and after the second moult.

Figs. 14–16. Right uropod of *C.* (*Bartonius*) *bartoni*, ♂, condition at amputation, before, and after, second ecdysis.

Fig. 17. Diagram illustrating the way in which the abnormal appendages shown in Figs. 1 and 2 may have been produced.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SEX RECOGNITION IN CYCLOPS.

S. J. HOLMES.

The sexual behavior of copepods presents several points of similarity with that of the amphipods which was described by the writer in a previous paper.[1] In both groups the males clasp and swim about with the females for a long time previous to copulation ; and in both groups the behavior of the female is much the same while being clasped by the male. Having an opportunity to study a thriving culture of *Cyclops fimbriatus* in which pairing was actively going on the endeavor was made to ascertain if the method of the sex recognition employed in the amphipods occurs also in this species of a quite distantly related group.

Male *Cyclops*, as is well known, have the first antennæ enlarged and modified to form a clasping organ. In *Cyclops fimbriatus* the male usually clasps the female just in front of an enlargement at the base of the abdomen. Females carrying eggs are sometimes seized, and also females not more than half grown. Males show great eagerness in grasping the females, and they can be compelled to release their hold only with difficulty. They may be poked about roughly with a needle and the posterior part of the body may be cut off without causing them to leave the female. I have often picked up pairs in a fine pipette and forcibly squirted them out several times without succeeding in separating the two sexes.

As the pairs of *Cyclops* swim through the water the males are usually the more active. Frequently the female remains entirely quiet with the appendages drawn close to the body, and the body flexed ventrally, allowing herself to be passively carried about by her mate. At other times the female may swim as actively as the male. In general the behavior of the females and their attitude while being carried closely resemble what is found among the Amphipoda. So also does their behavior when the males come in contact with them and attempt to seize them. The

[1] Biological Bulletin, Vol. 5, 1903.

female during the efforts of the male to clasp her around the base of the abdomen usually lies quiet with the appendages drawn close to the body. She may be seized by the legs, tip of the abdomen, or any other part of the body, but the male works around until he gets into his normal position which he sometimes attains only after much labor. Females vary greatly however in respect to their willingness to be clasped by the male, certain individuals resisting seizure for a long time.

So far as could be detected the males do not seek or follow the females at a distance as Parker concluded they did in *Labidocera*. The association of the sexes seems to be due merely to chance collisions. Males often attempt to seize other copepods with which they collide regardless of their sex. The males resist such attempts at seizure and dart quickly away, while the females often stop and submit readily to the clasping propensities of their companions. Several males were injured so that they could not resist seizure, and in many cases they were seized by other males who worked industriously until they got their burden clasped around the base of the abdomen in the usual way. These associations did not last long however; the active males apparently appreciating that something was wrong soon swam away. Recently killed females were often seized and in some cases carried about for a while, but they were finally dropped. Males seem rather more prone to seize dead females than members of their own sex. In one case I saw three males tugging away at a dead female, and they were soon joined by a fourth male who participated in the same effort.

It is possible that the odor of the female determines to a certain extent the sexual behavior of the males, but my experiments yielded no evidence of this. Several females were put into a tube one end of which was covered with fine gauze and the tube was then placed obliquely in water in which were numerous males. The males showed no tendency to congregate around the end of the tube where the females were confined. In another experiment several females were placed in a glass tube in which a small plug of loose cotton was inserted a short distance from one end. This end was laid obliquely in the water. The males showed no tendency to enter the open mouth of the tube as they

might be expected to do if they were attracted by the odor of the females. The experiment of removing the organ of smell which was performed in the case of the amphipods would be a fruitless one in *Cyclops*, as the seat of smell is located to a considerable degree at least in all probability in the same organs that are used for clasping.

It is evident that mating in *Cyclops* is brought about much as it is in the Amphipoda. The males have a strong tendency to clasp other copepods; the females tend to remain quiet in a condition somewhat resembling the death feint while being seized by the males. It is not improbable that olfactory stimuli may cause the males to remain with the females longer than they otherwise would, and they may render the males rather more prone to seize females than other males, but so far as could be determined by watching the behavior of the animals the specific reaction of the two sexes to certain kinds of contact stimuli is the main factor in bringing about their association.

OUR KNOWLEDGE OF MELANIN COLOR FORMATION AND ITS BEARING ON THE MENDELIAN DESCRIPTION OF HEREDITY.[1]

OSCAR RIDDLE.

Hardly a year has passed since the rediscovery of Mendel's Law without several additions to its descriptive terminology. This may signify either one of two things: a very healthy and vigorous growth, or the onset of senescence. Some of these newly introduced features are plainly justifiable; but there is reason to believe that the rather long series of extensions which has been made in recent times carries as its result not so much description of fact, as of deduction and far-reaching theory.

The facts and phenomena discovered by Mendel, and the array of facts of high importance which later workers in this field have brought before biologists, have already proved their value. The proved value of these facts is, however, no proof of the correctness of Mendelian interpretations of the processes of inheritance. I shall here present some *facts* which seem to indicate that these Mendelian *interpretations* are not sound; and further, that these unsound interpretations now stand as a formidable block in the path of progress to a better knowledge of the mechanism of inheritance and development.

Mendelian workers think that they have discovered, and certainly they have named and labelled, many "factors"[2] as necessary for the production of some single characters. These workers tie *all* of these factors together, and — for them — together they go into the germ cells, and whatever appears — or fails to appear — in the zygote is interpreted in terms of the

[1] Read January 19 before the Biological Club of the University of Chicago.

[2] The word *factor* as used here in a purely Mendelian sense represents quite a different thing from the physiological sense of the same word. The whole series of ordinary environmental factors — temperature, light, reaction of medium, concentration, moisture, etc., all these would not constitute even *one* Mendelian "factor." The Mendelian "factor" is often a rather unidentifiable thing, but it is conceived of as something capable of residence in, and of segregation by, the germ cells.

presence or absence (*dominance*, *inhibition*, *contamination*, etc., made use of by some workers) of *particular factors in the gamete*. But facts at hand, despite an opposite contention, will go very far towards showing that the method of analysis of the Mendelian worker has not permitted him to decide the question as to the *real number* and *separateness* of the factors; nor yet to determine as to whether certain of the factors were at all *represented in the germ cells*, or whether they may not have arisen during the ontogeny as a direct result of tissue differentiations, through regulatory processes, or otherwise, and quite independently of the existence of a definite determiner in the gamete or germ cells.

As Mendelism has developed, it has lent support to the doctrines of preformation, unit characters, and discontinuous variation. The facts and interpretations here brought forward disclose, on the other hand, no small amount of epigenesis, and strongly support the proposition that present and new knowledge will lessen, not widen, the apparent gap between discontinuous and continuous variability. There is, too, at present a marked tendency in some quarters to further elaborate and extend the "factor" hypothesis, which furnishes an additional and specific reason for my calling attention to some facts from my province of study which indicate that already we have represented too many factors in the germ cells; that qnite certainly some factors which have by Mendelian interpretation been made to circulate through the germ cells are never represented (in the Mendelian sense) in these cells at all; and finally that many factors considered most separate and discreet by Mendelians, can now be proved to be but points in lines of perfect continuity.

It will no doubt be urged by some Mendelians that the observations recorded here are quite wide of the mark because the writer has no experience in animal breeding. It is very true that I have not personally carried through any breeding experiments whatever. For information in this field I have depended upon what I have been able to see of the breeding and hybridization experiments conducted by others, and upon the literature of the subject. My own work for several years has been largely in the field of developmental and color physiology; its aim being to get

at the basis of the color characters of organisms. It must rest with biologists generally, however, to decide whether the facts here presented have, or have not, to do with the Mendelian interpretation and description of the processes involved in heredity and development.

The basis, then, of my objections to much of the Mendelian interpretation rests upon chemical and physiological facts regarding the origin and development of melanin pigments. It is necessary to anticipate the query as to how, or by what right, has melanin color formation anything to do with the essential points of Mendelism? I realize fully that the line of contact between these two provinces of activity is apparently not a line of contact at all, and so new and untrammeled is the territory that one would almost hesitate to enter, had not a pair of such good Mendelians as Cuénot and Bateson already knocked importuningly at the gateway which leads into it.

It should be recognized at the outset that, in thus presenting a body of facts from one field, as having important bearing on facts and theoretical deductions in another field, there is every risk that a short presentation will be incomplete, inaccurate, and at the same time may fail to properly or sufficiently orient the reader with respect to the writer's point of view. It is here impossible entirely to avoid incompleteness, inaccuracy, and but partial explanation of an opposed interpretation of the facts of color development and inheritance; it is hoped, however, that a presentation, and rather general though cursory discussion, of a limited number of facts — facts with which most biologists are not familiar, and which have never before been treated in this connection — will make it possible to recognize that some points of present biological theory are involved.

And, though many of my statements concerning such points of theory may seem dogmatic, I should like to make it clear that I am not deceived or blind to the fact that my present function is merely to introduce subject matter for a chapter, not to conclude a volume; to propose, not to decide. These discussions of theory would have been omitted entirely from this paper if it had been thought that the facts here brought for the first time into the field of heredity and developmental physiology would

receive the attention which they deserve without such a setting. I am, of course, rather confident of the correctness of the point of view set forth. I am absolutely convinced that the facts here presented will prove valuable assets to the student of development and inheritance.

There are three reasons why melanin color formation, better than any other process or group of processes, may furnish the starting point for certain inquiries and criticisms regarding the way Mendelian inheritance is construed and described:

1. Color characters have been more extensively studied and described from the Mendelian standpoint than have any others. A very considerable share of the color investigated — all mammalian color, for example — is due to melanin pigment.

2. It was to recognize a fact in melanin color development that Cuénot ('03) introduced the idea of *presence* and *absence* of a character, or character determiner, etc.; an idea which is now made by many workers to support practically the whole structure of Mendelian description and interpretation. Again, the now rather elaborate terminology introduced by Castle is based almost wholly upon the behavior of melanin colors. A few paragraphs of the paper by Cuénot furnish practically all there is of a tangible basis for representing *chromogens*, *enzymes*, and *activators* in gametic formulæ.

3. There is already at hand a certain amount of definite chemical knowledge, and some reasonably safe physiological information, which can be brought to bear on some points of the color philosophy of Mendelism. There is, moreover, something which though apparently less substantial, is none the less important — namely, the assurance of further, definite light from these same sources. There can be no doubt that we can use biochemical and physiological methods and data to give us what is now more needed than all else, perhaps, in the study of evolution and development — namely, *the intimate developmental history, and nature of some one character;* I mean the *proximate* history, the mechanics of what some would call the "late stages" of the development, or the "differentiation" of a character.

It may help to keep the reader oriented throughout this dis-

cussion to state that I shall first describe some of the facts of color development as they are known at present from chemical, pathological, and physiological experience ; and afterward sketch very briefly the nature of the Mendelian terminology ; this to be followed by some discussion of my point of view. Such facts of color will be considered as have bearing on the following points :

Do the known facts of the *genesis*, *nature* and *history* of color characters harmonize with, supplement, modify, or radically differ from, the demands of present Mendelian interpretation? Do they enable us to decide as to whether color characters are qualitative or quantitative in nature? Are color differences cases of continuous or discontinuous variability? Can these facts throw light upon the existence or nature of unit characters? What of the purity of gametes? Do these facts indicate a different or sounder basis for the interpretation of Mendelian, or other inheritance? What justification or light, if any, is thrown upon the present practices of (*a*) adding "factors" in order to account for the inheritance phenomena exhibited by a character ; (*b*) of tying all these "factors" together and postulating that all pass (by means of their representatives) through the germ cells?

Some Facts of Melanin Color Formation.

In a consideration of the facts of the origin of melanin coloration, one might deal at some length with the *distribution* and *histogenesis* of melanin. Though several interesting and illuminating facts lie in each of these directions, I shall dismiss these two phases of the origin of melanin colors with the single statement that the melanins are usually dark, amorphous or granular pigments, chiefly of intracellular, animal origin ; extending within this kingdom from the trypanosomes (Protozoa) to man. There is no vertebrate species (unless we may think of pure albinos as such), but has this coloring matter in one or several parts of its body. It is, however, the chemical and physiological phases of the origin of color that it is most desirable to discuss, and it is from this angle of approach that we find most of the facts which bear on the Mendelian description of heredity.

Our knowledge of what has been called the "mechanics of

melanogenesis" may be thought of as having begun with studies in the production of artificial melanins, and the accompanying search for the (melanin) chromogen in the albumen molecule. This work was shared by many workers: Stadelmann ('90), Gmelin ('94), Nencki ('95), Schmiedeberg ('97), Chittenden and Albro ('99), Hofmeister (see v. Fürth, '04), v. Fürth ('99, '01, '04), Hopkins and Cole ('01, '03), Schneider ('01), Samuely ('02) and others. Through these workers it was made known, first, that melanins artificially produced are essentially the equivalents of natural melanins; and second, that tyrosin and related aromatic compounds are the chromogens concerned.

The second step in the progress of this knowledge was concerned with the nature of the process by which the melanin is formed from the chromogen. Hlasiwetz and Habermann ('73) had first recognized oxidative processes as necessary for the formation of the artificial melanins. Landolt ('99) extended this fact to the natural pigment of the choroid.

Bertrand then discovered ('96) an oxidizing enzyme — tyrosinase — which was able to transform tyrosin into melanin-like bodies. Bertrand found the enzyme in certain plants. It has since been found to be of wide distribution, having been found by Biedermann ('98) in the contents of the alimentary canal of meal worms; by Lepinois ('99) and Gessard ('01) in the adrenal glands; by Gonnermann ('00) in beet roots; by v. Fürth and Schneider ('01) in the hæmolymph of insects; by Przibram (see preceding, '01) in the ink-sacs of cephalopods (*Sepia*); by Ducceschi ('01) in the blood of *Bombyx;* by Gessard ('02*a*, '03*a*, '03*b*) in the ink-sacs of *Sepia*, in the integuments of insects, and in melanotic tumors of horses; by Dewitz ('02) in the blood of certain insects; by Durham ('04) in the skins of mammals and birds; by Weindl ('07) in the skin, eyes, ink-sacs and eggs of *Loligo;* and by Bertrand and Mutermilch ('07) in wheat bran. v. Fürth and Schneider ('01) concluded that "tyrosinase-like ferments are widely distributed in the animal organism, and probably always appear wherever and whenever a physiological or pathological formation of melanin occurs."

Meanwhile, another advance in our knowledge of melanogenesis was made when Dewitz ('02) demonstrated the rôle of an

oxidizing enzyme (tyrosinase) in the normal development of the dark pigment of the integuments of living, growing animals (fly-larvæ — *Lucilia Cæsar*). At the same time he was able to prove that, in the forms with which he worked, free oxygen is also an indispensable factor in the development of the color. This work, important and suggestive as it was then, is now made still more valuable by new knowledge of the chromogen — that is, the other factor involved in the pigment formation. Without knowing just what this chromogen might be, Dewitz was able to conclude (p. 45), "We cannot doubt that we have here in the blood of the larvæ an enzyme under the influence of which a chromogen is oxidized and forms a brown or black pigment."

A year later Gessard ('03*b*) was able to show that in the melanotic tumors of white horses not only tyrosinase but free tyrosin is present. He concludes (p. 1088): "Tyrosin is then the chromogen, the oxidation of which by tyrosinase determines the formation of the black pigment which is common to many physiological and pathological products of the animal economy; and it can be said that the color of the negro is due to the same reaction that produces the ink of the squid, or the black color of some mushrooms." Gessard states, too, that when tyrosin is oxidized with tyrosinase it gives a series of colors — "rose, rouge-grenat et brune." In a later work ('03*d*) he made a closer study of the color reactions of tyrosin in which he showed that the presence of acids, alkalis and salts have marked effects on the colors produced.

The recent work (May, 1908) of Bertrand, is, however, of the highest interest. He has been able to determine (1) the *type of substance — of which there are many representatives — which can by the use of tyrosinase be oxidized to melanin compounds;* (2) *he has shown that each one of these compounds passes through a series of colors before arriving at the final stage of oxidation*; (3) *that this series varies somewhat as to the exact tint of the initial and final colors*, but that (4) the *early stages of oxidation uniformly give lighter colors than the later ones*, the series usually running from yellow to orange, through darker tints to brown or black.

Bertrand's studies make it clear that any benzene nucleus with an attached hydroxyl can be acted upon by tyrosinase and converted

into melanin pigment. Thus the whole series of compounds in the table given below (and many others besides) can be oxidized to colored compounds. On the other hand, phenylalanine, phenylmethylamine, phenylominoacetic, phenylpropionic and phenylacetic acids, alanin, glycocoll, etc., give no coloration whatever. The size, complexity, and nature of the lateral chain has only a subordinate influence ; if it is not very strongly acid or basic it will not interfere with the oxidation. Thus for example, ethyltyrosin, chloracetyltyrosin, and glycyltyrosin were oxidized with ease. The nature of these side chains does, however, *considerably modify the colors produced* by the oxidation. Neither of the three last named bodies (tyrosin compounds) give a final black color ; they begin with orange or yellow and end with red or mahogany (chocolate ?).

In order to further appreciate something of the variety of color which may arise from a *single chromogen*, and to get an introductory idea of the number and variety of chromogens to be found in the animal body, careful reference to Table I. should be made. *And here it is of the highest importance to see that a single chromogen acted upon by a single enzyme* (so far as all chemical experience has detected) *produces several colors depending upon the degree of oxidation involved.*

In regard to the rate at which these colors appeared the author's statement may be cited that in a 20 per cent. tyrosinase (80 per cent. strong tyrosin) solution, tyrosin developed a rose color in ten minutes and its black color in four to five hours.

TABLE I. (From Bertrand, '08.)

Name of Body.	Colors Produced by Oxidation.
Tyrosin	red grenadine, then inky black.
p-oxyphenylethylamine	red grenadine, then black olivaceous.
p-oxyphenylmethylamine	orange yellow, orange red, maroon.
p-oxyphenylamine	orange, mahogany red, then brown.
p-oxyphenylpropionic acid	orange yellow, grenadine red, brown.
p-oxyphenylacetic "	yellow, orange yellow, then brown.
p-oxybenzoic "	(weak) rose, orange, then yellow.
p-cresol	yellow, orange, then red.
Phenol	yellow, orange, red, then brown.

These are, from our present point of view, the more notable results of Bertrand's investigations of what he calls " the mechan-

ism of melanogenesis." This author does not in any way consider the bearing of his findings on Mendelian descriptions of the mechanism of the origin of melanin *color characters* (neither on any aspect of inheritance). Nor — as previously stated — has anyone, at any time, inquired as to whether the facts obtained in the former sphere are compatible with the assumptions made in the latter. Bertrand's studies had other and quite different objects; these were: First, to learn the degree of specificity of the enzyme tyrosinase; the conclusion here being (p. 387) "that the results speak once more against the principle of very absolute specificity which one nowadays often hears applied to enzyme actions." It will be very well for us to bear in mind this result, since, as we shall see, Mendelian description demands a still higer degree of enzyme specificity than the philosophy of biochemists has yet dreamed of. A second object of his work seems to have been to consider the possibility of identifying certain of these tyrosin bodies by the color reactions they give upon oxidation with tyrosinase. A third purpose of the study was concerned with the causes of the rather wide variations in the elementary composition of different melanins; he believes that the results give some reason for believing that the simplest melanins arise from the oxidation of tyrosin itself, while the more complex ones — those containing sulphur or iron — are formed by the oxidation of less complete products of protein hydrolysis — namely, tyrosin-containing di- or polypeptids.[1] A fourth and final phase of Bertrand's investigation touched upon a hitherto unrecognized, but seemingly possible, mode of union between tyrosin and other amino acids.

It will now be well to briefly sketch a few facts obtained from the study of abnormal tyrosin metabolism, and from pathological pigmentations (melanin) of the human body, as supplementary to the account of melanogenesis which is given above. These facts will also serve to illustrate the dependence of tyrosin oxidations upon somatic conditions which may be of such a temporary, intermittent, quantitative, or reversible character as to preclude

[1] In the formation of melanins, condensations as well as oxidations occur, but the former process need not concern us in treating the present theme.

the possibility of accounting for them on the basis of specific, independent transmissions, once for all segregated by the germ cells.

Rather fortunately for the completer view of our present theme, pathologists and clinicians have been frequently confronted with cases of incomplete tyrosin oxidation (alkaptonuria), and unusual and pathological melanin pigmentations (melanotic tumors, Addison's disease, ochronosis) in the human body. These subjects because of their medical bearings have received a very great amount of attention, of accurate and searching study, at the hands of investigators. It seems self-evident that the student of melanogenesis should here find much data to interest him.

In the condition known as "alkaptonuria"[1] the alkapton acids — uroleuic and homogentisic — appear in the urine. The last-named compound represents a stage in the oxidation of tyrosin. The intermediate stages and the chemical structure of these several compounds may be best understood by reference to Table II. Our interest in these early stages of tyrosin oxidation is very great since we know that the same, or similar steps, lead in special cases to the formation of melanin.[2] Neubauer ('08) has very recently determined the exact course of the first four steps of tyrosin oxidation as they occur in the living (human) body. The chemical expression of these stages is given in the table.

Garrod ('02) found that certain individuals, who in their youth excreted urine containing homogentisic and urolencic[3] acids, produced only the former during adult life. Other cases of temporary and intermittent alkaptonuria are known. Here certainly the evidence of our senses is simply that the *power of the organism to oxidize tyrosin compounds is not dependent primarily upon germinal segregations*, but rather upon tissue activities, relations

[1] Résumé and literature by Falta, *Biochem. Centralb.*, 3, p. 174, 1904. See also Abderhalden, "Lehrbuch der Physiologischen Chemie," Berlin, 1906, pp. 294–298; and Neubauer ('08), *loc. cit.*

[2] A chemical research directed to the determination of the reasons for some tyrosin oxidation leading to melanin formation, instead of to the usual end-products of oxidation — NH_3, CO_2, H_2O, etc., would be of the greatest interest and help in studies on the physiology, development and heredity of color.

[3] According to Neubauer ('08) this body is not an intermediate step in the oxidation of *tyrosin* to homogentisic acid.

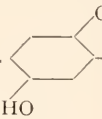
Table II.

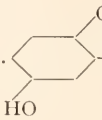
Showing Some of the Known Facts of Chemical Experience Concerning the Succession of Oxidations of Tyrosin and Closely Related Bodies to Melanin Pigments.

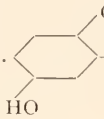
Tyrosin = ...HO—  —$CH_2CH(NH_2)$—COOH[1] Colorless.

P-oxyphenyl-pyrotartaric acid = HO—  —CH_2—CO—COOH[1] "

Chinol = ... O=  —CH_2—CO—COOH[1] "

Hydrochinon-pyrotartaric acid = ...  —CH_2—CO—COOH[1] "

Homogentisic acid[4] = ...  —CH_2—COOH[1] "

Gentisic acid = ...  —COOH "

Melanogen .....( ) ..... "

Melanin.....( ).....(White)?[2]

Melanin.....( ).....Pale yellow.[3]

Melanin.....( ).....Deep yellow.[3]

Melanin.....( ).....Red.[3]

Melanin .....( ).....Brown.[3]

Melanin.....($C_{50}H_{58}N_8SO_{12}$).....Black.[3]

Melanin.....($C_{45}H_{78}N_{10}SO_{20}$).....(White)?[2]

and conditions; these may vary or change from year to year—a power of oxidation not possessed by the individual during many early years of life being attained at manhood, or vice versa. Bateson ('02) has seen fit to claim that (p. 133, note) alkaptonuria is the result in inheritance of the union of "two recessives." Garrod has concurred in the view. But in the light of the inconstant and intermittent character of the phenomenon, it seems necessary to draw a directly opposite conclusion.

[1] This according to Neubauer ('08).

[2] See discussion of Spiegler's work ('03), etc., p. 328 of this paper.

[3] These from Gessard ('03) and from Bertrand ('08).

[4] Concerning the formation of homogentisic acid from tyrosin in plant tissues, see Schulze and Castoro, *Zeit. f. Physiol. Chem.*, Bd. 48, p. 396, 1906.

Examples of other temporary or intermittent oxidative powers might be much extended to include cases of glycosuria, cystinuria, purin metabolism, etc. I shall not discuss these cases which have only an indirect bearing on our question of tyrosin oxidation. It is, however, of some interest to state that it has become evident through the work of Abderhalden and Schittenhelm ('05), Garrod and Hurtley ('06), and others, that the body may possess a low oxidizing power for *several different protein constituents at the same time;* as, for example, in some cases of cystinuria, when diamines, tyrosin, lysin, etc., in addition to cystin, pass through the body unoxidized and appear as such in the urine.

The known facts of abnormal pigmentations deserve a larger share of attention than they receive here. They are mentioned chiefly to direct attention to a field of facts that are quite completely ignored in our theories of the heredity of color.

In the condition known as *ochronosis*, certain cartilages (*e. g.*, those of the ear) and connective tissues become pigmented. The work of Albrecht ('02), Osler ('04), Pick ('06), and others, make it certain that ochronosis is a form of melanotic pigmentation, and that it is not uncommonly associated with melanuria, or alkaptonuria and even with the pigmentation of the sclerotics and skin (Osler). Similarly, in *Addison's disease* there is deposited in the skin a pigment which, according to Pforringer ('00) differs from that produced normally only in quantity and not in origin or composition. It is well known too that *nerve lesions* are often accompanied by pathological pigmentation of the skin.[1]

A word in regard to *melanotic tumors*. These are known to occur particularly in white horses. The amount of melanin produced is often very great. Abel and Davis ('96) estimate the melanin of the entire skin of a negro at 1 gram, whereas Nencki and Berdez ('85) found 300 grams of melanin in a sarcomatous liver, and estimate that the entire body contained 500 grams.

These several facts from pathology are significant in that they indicate that *for the building of any melanin at all, the actual local conditions of the organism, or the organ, have a rôle to play that*

[1] See résumé by Schmidt, *Ergeb. der Pathol.*, Bd. III., Abt. 1, p. 551, 1896.

is quite out of keeping with any "once for all determination" by the shuffling of color "factors" through the germs.

In this connection attention should be called to the fact that Spiegler ('03) has reported the finding of a *white* melanin in the white hair of horses, and in sheep's wool. It would seem that the melanin isolated by him represents a more advanced stage of oxidation than does black melanin. If this is true it is obviously an important fact. The isolation of a similar pigment from the white hair of *albinos* seems, however, neither to have been sought for, nor found; but it is quite possible that such a pigment also exists in mammalian albinos. There are nevertheless some reasons — chiefly biological rather than chemical — for believing that among birds, at least, a white color exists which represents a less advanced stage of oxidation than that in any other of the melanin color series; in fact, here the "white" seems to be a purely "physical" color. The chromatophores do not develop, and no white pigment is histologically discoverable. Apparently, therefore, the oxidation of tyrosin, etc., is here not carried far enough to produce any color whatever.

The actual facts regarding the "white" pigment or color of birds and mammals are not yet clear. "White" forms at present a most awkward, and at the same time a most interesting gap in our knowledge of the melanin series. The elementary formulas for the black and white pigments given in the table are Spiegler's findings for the white and black hair of the horse. Certain phases of Spiegler's research have been confirmed by Wolff ('04), but the particular facts which we have referred to above have not been reviewed or confirmed.

We are fortunate in having, from physiological experiments with melanin pigments in living animals, some facts which confirm the data from chemistry and pathology regarding the mechanism of melanin production. On this point special attention may be called to the recent work of Gustav Tornier. His work furnishes a splendid view of the *control* of the color of the integument. The colors of Amphibia, from larval stages to old age, were determined at will by controlling the physiological state, particularly the nutrition, of the animals. When the color

phenomena observed by Tornier are viewed in the light of the work of Bertrand, it seems certain that in these two sets of phenomena we are really dealing with the same facts.

Tornier found that tadpoles divided into lots for differential feedings gave (1) little or no pigment in the ones fed the minimum, and progressively more pigment as the maximum is approached; (2) *a series of colors: white, yellow, red, gray, black.* Tornier concludes ('07*b*, p. 288): It is possible, therefore, by adjusting the dosage of fleshy food to force the epidermal coloration of *Pelobates* larvæ (he elsewhere describes this as true for other amphibia) into white, yellow (see p. 285), red, gray, black.[1] It will be seen that the types of color and the order of their appearance in the organism — when we put these organisms into such conditions as will force them to do the work of pigment formation (oxidation) in stages — closely follow the lines of our purely chemical experience. Tornier produces entirely comparable effects upon the coloration by two other means, viz.: by removing more or less yolk from the vegetative pole of the egg through an opening made by a needle; or by coagulating *in situ* a part of yolk proteid by the introduction of water; such coagulations of yolk proteid cannot be digested by the developing embryo. The three methods employed all reduce the nutrition of the animals, and produced albinism, erythrinism, blackness, or melanism, depending upon the state of nutrition.

It was further found ('07) that the experiment could be carried out in the *opposite direction* as well; that is to say, the highly fed, black-containing, and black-producing larvæ of large or small size could be made, through a diminution of feeding, to produce a *series of colors in the order of: black, brown, red, gray, white.* Certain observations by Powers ('08) are confirmations of Tornier's [1] results.

Without extending these illustrations it can be stated that if these facts and experiments mean anything they mean that in an animal that produces melaninic color, *there exists all the machinery necessary to produce a series or scale of these colors.* And that

[1] The proof that all these shades of color in Tornier's tadpoles were melanins is hardly as complete as is desirable, since lipochromes and traces of guanin are also known to develop normally in late larval and adult stages of these amphibia. Many facts, however, indicate that the colors here described by Tornier are true melanins.

what is actually produced is, in several demonstrated instances, *dependent upon the physiological state of the organism.* Or, perhaps, in certain cases it may be possible to say more definitely that the *limiting factor* is none other than the available oxygen or food-supply. I have been able to prove ('08*a*) definitely that in many birds the daily nutritive changes which accompany the low blood-pressures occurring at night influence the *quantity* of melanin produced.

The specific color of an animal then is an index, not of the presence in the germ from which this animal arose, of certain chromogens and specific zymogens, and the absence of a wide series of others; but, this specific color means that a *process* with a wide range of possibilities, *because of a particular physiological state and environmental conditions* has struck this particular equilibrium. *One and the same organism has within it all that is necessary to move that equilibrium up or down* — taking the red form for example, we can in the words of Tornier "force it to black or to white."

Tornier did not consider the relation of his results to any of the facts of the chemistry of melanin, nor did he consider their bearing on color inheritance. His chief concern has been apparently to establish two points in color physiology; first, the effect of varying degrees of nutrition on the size, shape, color-production, etc., of chromatophores; and, secondly, a defense of the thesis that the pigment granules of these chromatophores act as reserve food-materials in cases of inanition, etc.

Mendelian Description.

It is now possible to consider whether Mendelian interpretation and description is in accord with the facts of color formation. The Mendelian position can be best presented in the words which Cuénot ('03) used in the original formulation and statement of it. I quote practically the whole of his discussion, and ask that it be remembered that it is almost entirely upon this slender basis that the "presence, absence" hypothesis, and consequently a great share of Mendelian nomenclature, rests:

"Again one learns that the authors who have recently studied the origin of melanin pigments, Biedermann, v. Fürth, Schneider

and Gessard, state that these pigments result from the action of an oxidizing enzyme (tyrosinase) upon a chromogenic substance ; there are good reasons for supposing that things happen similarly in the pigmentation of the skin; there should be, however, in this case, either two different chromogens and only one enzyme, or only one chromogen and two enzymes, the one for the blackish pigment and the other for the yellow pigment. We adopt provisionally, for convenience of language, this latter hypothesis.

" The germ plasma of a gray mouse should contain potentially the three substances which, by their reciprocal reactions later produce the deposition of pigment in the hair; and doubtless these three substances are contained in the potential state within many of the material particles of the germ plasma (representative particles or qualitative substances of the egg — mnémons). In a gray mouse (black and yellow pigmented) there are three mnémons, one for the chromogen and two for the two ferments ; in a black mouse there are only two mnémons, one for the chromogen and another for the formative enzyme of black pigment.

" In regard to albinos, all is explained if we admit that their germ plasma contains only the mnémons of the enzymes, that of the chromogen being totally absent. With these conditions, colored hair cannot be formed in albinos, since one of the substances indispensable to the reaction is absent, but one easily understands that the albino will transmit to its progeny either the mnémons for the two enzymes, or one mnémon only, if it possesses but one."

The Mendelians have one further point to confirm the faith that is in them. Soon after the appearance of the paper by Cuénot, Durham undertook to find whether in the skins of black, chocolate, yellow and albino mammals there is the appropriate enzyme in each for the production of its particular color — when this acts upon a tyrosin solution. Only a short preliminary statement ('04) of the results has appeared ; and although positive results were reported for the black, chocolate and yellow pigments, it is evident that from no point of view can these results be regarded as satisfactory; particularly because the extracts used by her are stated to have had a *reddish* color before

adding the tyrosin; an adequate account of the history or nature of color changes in such a solution seems hardly possible since as Table I. shows much of melanin production results in colors which are paler than red and the origin of all these, and to some extent the final color, would be obscured by the initial presence of red. Miss Durham was unable to state exactly what the extracts of the albino skins were capable of doing, but thought they probably contained no such enzymes.

The factor hypothesis of Castle avoids some of the pitfalls of the earlier theory, but seems to rest on essentially the same base. It is necessary to examine in detail the statements and conclusions in Cuénot's paper.

Cuénot says: "There should be, however, in this case either two different chromogens and only one enzyme, or only one chromogen and two enzymes, the one for the blackish, the other for the yellow pigment." The facts of the origin of melanin do not substantiate Cuénot's hypothesis because the colors do not form on this plan; one and the same chromogen is known to form yellow and black; one and the same ferment — tyrosinase — is known to produce both yellow and black from the same chromogen.

According to Cuénot: "The germ plasma of the gray mouse (black and yellow pigment) should contain potentially the three substances which, by their reciprocal reactions, later produce the deposition of pigment in the hair; and doubtless these three substances are contained in the potential state within many of the material particles of the germ plasma (representative particles, mnémons, etc.)." But three such substances are not required for the production of black and yellow; these two color compounds are known to be but different stages of oxidation of the same substance. Furthermore, the locating of all the factors which determine the development of these colors in the germ plasm does not reckon with the facts of color physiology already cited.

Continuing, Cuénot says: "In a gray mouse (black and yellow pigmented) there are three mnémons, one for the chromogen, and two for the two enzymes; in a black mouse there are only two mnémons, one for the chromogen and another for the formative enzyme of black pigment." Statements made above furnish a sufficient refutation of this conception of Cuénot's.

Cuénot's concluding statement: "In regard to albinos all is explained if we admit that the germ-plasma contains only mnémons of the enzymes, that of the chromogen being totally absent. With these conditions, colored hair cannot be formed in albinos, since one of the substances indispensable to the reaction is absent, but one easily understands that the albino will transmit to its progeny either the mnémons for the two enzymes, or one mnémon only if it possesses but one." To this statement must be opposed first the opinion of Durham that the skins of the albino mammals studied by her contained no tyrosin-oxidizing enzymes; a second much more weighty and conclusive objection is that the absence of melanin chromogens in albinos is practically inconceivable. It is now certain that tyrosin and its many related compounds are such chromogens, and that these compounds have a distribution in the universe almost or quite co-extensive with protoplasm itself. *The postulation of the formation of chromogen-containing, and non-chromogen-containing gametes is therefore reduced to an absurdity. It is moreover quite certain that the food of the albino mouse must daily bring it quantities of chromogens, even if such could have been excluded from the germ cells. There is no doubt and no middle ground here. Cuénot's conception fails completely.*

Space does not permit a discussion of the facts and interpretations of the inheritance of coat colors of mice since the work of Cuénot; a subject which has been investigated or discussed by many workers, notably by Bateson, Allen, Cuénot, Morgan, Wilson, Castle and Durham. A detailed consideration of these results is omitted also because the facts are well known and do not belong here. The behavior of the color yellow first reported by Cuénot ('05) is, however, of such unusual interest as to deserve special mention. It was found that yellow is "dominant" in mice, whereas elsewhere in animals it is usually "recessive" to black; this is an unexpected result from the Mendelian standpoint, and the difficulties which it presents have called forth several highly complex, supplementary Mendelian hypotheses. It seems not to have occurred to anyone that yellow may be a *blend* formed from the union of other colors, *e. g.*, between albino and black. A glance at our scale of colors (p. 326) sug-

gests that such may be a true, and, indeed, the truest conception of a color blend; and an examination of the experimental results gives considerable support to this view. Castle ('06) reports that he secured yellow mice from three sources, viz.: Y♂ × Ch ♀, A × B, A × Ch. It will be observed that each of these crosses has one color (yellow, chocolate, black, albino) *more* oxidized than yellow, and one *less* oxidized than yellow (see Table II.); that is, the yellow produced in these cases is apparently a blend.[1] The general fact of the unstable (heterozygous) character of all yellow mice is, quite possibly, evidence of the same kind.

Heretofore a blend, say between white and black, has been considered a mixture of these two colors, a spotted animal, a form in which black was diminished, etc., but a little reflection upon facts already stated concerning the nature of these color characters reveals *very distinct colors as none the less very distinct blends!*

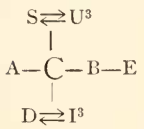
At present the *biological* data are wanting to quantitatively seriate all of the several colors; but there is apparently enough data to warrant the definite statement that *yellow mice* are forms with the power to oxidize tyrosin compounds to an *intermediate point.* Thus the biological data again parallel chemical experience.

Cuénot, Bateson and others "explain" color inheritance on the assumption that "recessives" lack altogether the *factors* of color production; but Castle has convincingly argued that this cannot be true, because in such forms *small amounts* of pigment actually form, etc. Castle tried to explain these phenomena upon the supposition that all of the factors may be present, but that "the presence of one character often inhibits the activity of another," *i. e.*, upon grounds of activity and latency. I would urge that we are now quite ready to take the next step, which seems to lie in the opposite direction, and say that we have to do neither with absent factors nor with the inhibition of present factors; that in gametic unions we deal not at all with "factor" particles, but merely mix, and amalgamate to various degrees, powers of tyrosin oxidation; and the conditions supplied by the differentiation of

[1] Cuénot says of his yellow mice that they contain numerous unfixable variations, not hereditary, ranging from a clear orange yellow to a sooty or grayish yellow, not very different from the color of gray mice. Does this look like purity of gametes, or a wide range of blending, which?

tissues and organs, together with environmental conditions external and internal, supply whatever else is concerned in color production.[1]

In his work on mice Castle ('06) shows conclusively that in these forms he is not dealing with the *total absence of a character*, even in some cases where this seems apparent; he there states that the *purity of gametes* does not exist, and further, "no more does the *purity of factors* exist." It would seem that this paper by Castle is one of the best, if not the best document extant to convince that no such things as *factors* exist. Yet, quite recently,[2] Castle has carried the factor hypothesis to its highest state of complexity, to what is apparently its logical conclusion, if any "particle" basis whatever could be granted for hereditary processes. Castle pictures his conception of the factors in melanin color inheritance and their relations in the following diagram, modelled after a chemical formula:



C = Color, or chromogen factor.
A = Agouti factor.
B = Black "
D = Dilution "
E = Extension factor.
I = Intensity "
U = Uniformity "
S = Spotted "

This visualizes for us the body of factors which are to be shuffled in the germs and "determine" the colors of the progeny. From what has been said it is obvious how far this conception leads us astray; because this complex Medelian interpretation and description of color inheritance leads us away from a simple series of color developments due to the difference in degree to which one substance may be oxidized.

The placing of the "uniformity-spotted," "intensity-dilution," and "extension" factors in these germs is a virtual surrender of the whole theory of discontinuous variation; and in reality puts

[1] The many and accumulating additions, qualifications, "contaminations," "latencies," etc., that have been attached to Mendel's ('65) original conception of dominance and recessiveness, or to Cuénot's ('03) presence and absence of a factor hypothesis, are but so many direct admissions that the "purity of gametes" conception is an error; they are but so many secondary and tertiary hypotheses of completer preformation made to bolster up a primal preformation hypothesis. Incidentally, they give present-day students the opportunity to see the child of Weismannism recapitulate the developmental history of the parent.

[2] Darwinian lecture, Baltimore, January 1, 1909.

[3] Either one of the pair may be present (or active).

the Mendelian who accepts this terminology in no position to deny the development of the melanin series on a basis of closely graduated powers of tyrosin oxidation, and so without any basis on particles or factors, whatever. It is, moreover, as plain as it is certain, that this *degree of oxidizing power* covers several of these factors (A, B, E, I, D) which are thus reduced to one; while the kernel of this formula the C, or chromogen, goes out entirely as a factor, *i. e.*, something capable of being shuffled in the germs — since as we have pointed out, such a chromogen is universal in protoplasm.

Discussion.

If the later oxidations — those which produce color — of the tyrosin compounds are each individually controlled by a separate, specific enzyme, why are not the several earlier oxidations of the same compounds similarly conditioned? If assumption will give us the whole series of tyrosin oxidations only on condition of their production by means of separate and distinct enzymes, why should it hesitate to put the whole vast array of oxidations of all aromatic, or even of all organic compounds on a similar basis — a separate and specific enzyme, separately heritable, for each step in oxidation? This is certainly not true.

It is impossible at present to announce the limits to the specificity of enzymes, and of the oxidases generally; but it can be said that it is pretty generally conceded that the oxidases present less specificity than do the digestive enzymes (see Wilcock, '06). That which weighs most heavily against the Mendelian assumption of a high specificity of tyrosin oxidizing enzymes is, however, the result of Bertrand's special study of tyrosinase which indicates no such specificity (quoted p. 324 of this paper).

But granted the greatest possible specificity of these enzymes, the Mendelian description of color inheritance becomes even more untenable; for Gessard and Bertrand have shown that "black" is the end-result of a series of successive oxidations and this final color can be attained only by having *all* of the intermediate stages actually attained. This means that the animal that transmits the enzyme for black, *i. e.*, produces black colored offspring, must *at the same time* transmit also the enzyme

for *brown, chocolate, red, yellow, etc.* (more accurately, an enzyme for each step of oxidation from tyrosin to black melanin), *without the absence of a single one.* If there be introduced here the primal Mendelian conception of the freedom of *each of these* factors to be distributed in gametogenesis according to the laws of chance, how often then may we expect pure-bred black parents to produce black offspring?[1]

There are reasons, derived from our general knowledge of oxidizing enzymes, why this assumption of high tyrosinase specificity is highly improbable and some evidence against this position has been cited from Gessard and Bertrand; a direct refutation of it is furnished by the experiments of Tornier. He showed that he could take animals which would have, according to Mendelian assumption, the enzyme for "black," and make them produce any one of three or four of the less oxidized members of the color series; these same forms could again under other conditions be made to produce the more highly oxidized black, etc. Obviously, the presence of a black-producing enzyme did not determine the color here; but conditions of life did so determine. The further assumption of inhibiting factors ready at all points of color-production to account for lower grades of color formation, and all other secondary assumptions to support the primary one are clearly unnecessary, and since a clearer, saner interpretation is possible they need not be considered.

[1] This would be the usual or expected type of specificity if such thing should exist. I call attention to its implications merely to forestall any further thought regarding its possibility. The sort of specificity of enzymes that has thus far been assumed by the Mendelians has, however, been of a different sort; namely, that for the production of each color only one enzyme is necessary, but the enzyme which produces any particular color is specifically different from those which produce other colors; the case is not really different from an assumption that pepsin is specifically different in different, but closely related races and varieties. Unlike the case cited above, here each germ possesses only one or two zymogens, each capable of supervising the complete production of some one color, and therefore all the offspring could (in contrast to above) be provided with color. The only evidence that has been adduced for this sort of specificity is the rather incomplete and unsatisfactory results of Miss Durham already cited. All else has been mere assumption on the part of the Mendelians. But this type of specificity becomes a very unusual and extraordinary thing as soon as we find that all of the colors form in a continuous oxidation series. To cover this fact the assumption is obligatory that in melanin production, six, ten or a dozen tyrosin oxidizing enzymes are concerned; that these are all able to take the first steps of tyrosin oxidation; that they are differentiated merely by their "strength," that is, the extent to which they can carry the oxidations.

The doctrine of numerous specific enzymes,[1] then, goes the way of the doctrine of specific *chromogens*, which is so decisively settled by the work of Bertrand.[2] Remembering that the Mendelian description (by implication) of what is happening on the (color-producing) surface of the body in late stages of development is thus completely awry, we may not be surprised to find that their assumptions regarding conditions in the germ are in a similarly contradictory tangle.

Our present knowledge permits neither the realization nor the imagination of a "color factor" in the germ; not even in a simple form; much less does it grant us the very composite and elaborate picture presented by Castle.

The "production of color" is a special manifestation, in rather restricted regions of an organism, of a *general power to oxidize organic compounds, possessed, presumably, by all parts of the germ cell* from which the organism arose. With the development of the body, the specialization of tissues, there arise very new environments for the oxidative processes, producing localized changes and variations in this power. That this is so is evidenced by the fact that the living substance of the various body regions oxidizes fats, sugars, and proteids with unequal ease. There can be, moreover, scarcely a doubt that certain of these regions, owing to new structure, new environment, new conditions, are able to oxidize *different protein substances* with variable ease, and to a variable extent, and even in a different way.

When one has grasped the nature of the process by which melanin color characters are formed, there is as little necessity or truth in assuming that the germ cells contain representatives or determiners to correspond to a particular color, as there is in assuming that the vapors of the Gulf contain determiners for the depth, or distribution, of the mantle of snow which they are to

[1] Mendelians must, however, accept the specificity or non-specificity of enzymes in the production of color (they must have some sort of representative particle in the germ) and either choice leads, when critically examined, to the unqualified refutation of some of their fundamental conceptions and interpretations of heredity. The established fact that each of the melanin colors represents but a point in a line — a line which records the continuity of a continuous oxidation process — is the fact that strikes hard at the very basis of Mendelian interpretation and description.

[2] Table I. shows, for example, that at least seven of the nine compounds represented are capable of producing the colors yellow and red.

form on the northern hills. There exists, to be sure, a relation between the vapors and the heaps of snow, between the egg and the definitive character — but the one is not the other, does not *contain* the other.

In accounting for melanin color characters, I would maintain that — granted the continuance of other life and developmental processes — we can account for all the major happenings of color development and inheritance with extremely little of assumption. It is *known* that one oxidase is concerned. I think we need make use of but one. It is *known* that the germ possesses actively the power to oxidize amino acids. I think we need make use of nothing in the germ than just this power. It is *known* that the protoplasm of different species, of different tissues, of different parts of a cell, possess different powers of oxidizing protein bodies. It is no tremendous assumption that germ cells are not freaks of nature in this respect, and that they too have different powers of tyrosin oxidation. The fate of color characters then is bound up (1) in the union of these particular powers of the two germ cells; (2) in the origin (through other outside developmental processess) of favorable and unfavorable regions for tyrosin oxidations; and (3) in environmental conditions.

Let us now for a moment return to the matter of color-blends. The data given furnish a nearer view — practically a new conception — of the nature of color-blends in inheritance; if the position stated in regard to these color-blends is correct a little thought will convince that at the same time new light is furnished on *alternative* color inheritance; and particularly on *what is happening* in the case of the so-called alternative (Mendelian) inheritance of a color character. It can be said definitely in such a cross that it is the *power to oxidize tyrosin compounds* (a power which I believe, by no stretch of imagination, needs to be, or can be, represented by a particle in the germ, but by a general property of the protoplasm of germ cells and of tissue cells) *that is transmitted* and that here this power of *one* of the gametes *is continued into*[1]

[1] Tyrosinase has recently been found in the *eggs* of cephalopods by Weindl ('07). Similarly, several enzymes have been isolated from germ cells in recent years; in none of these cases can we for a moment suppose that these enzymes or zymogens existed as *a* particle in any one, or in each, of the chromosomes. I would, however, by no means have it inferred that I consider the presence, quantity, etc., of tyrosinase

the zygote without being very *appreciably* increased or diminished.

It may be, however, that more than one (almost certainly several for yellow) oxidation stage of a tyrosin compound presents only the one color black. It could conceivably (see scale of colors, p. 326) happen, therefore, that a certain black (low stage of oxidation) × light yellow = yellow (dominant?); but another black (highly oxidized) × yellow = black (dominant?); *and yet each of these might be true blends, i. e.*, attain to an exact intermediate stage of oxidation to the two forms crossed. Some cases of supposed dominance of color may be therefore in reality true examples of blended inheritance.

In describing color inheritance I believe that less violence is done to the known facts of color formation, and at the same time a sounder view of developmental and hereditary processes is maintained, if it be said — without delimiting terminology, and without putting a single thing into the germ except what every one knows is there, and there in the form which is stated for it — that in the union of germ cells derived from two pure color varieties, each cell brings with it a power of oxidizing tyrosin compounds, and that the union of the pair of cells may give one or more of the following results:

1. The tyrosin oxidizing power of the male cell is established (*a*) at once, or (*b*) in the next generation or later, throughout the fertilized ovum and its derivatives.

2. The tyrosin oxidizing power of the female cell is so established. (In neither of these cases do we need to postulate the continued existence of a subdued — recessive — factor or representative.) These would be so-called dominants.

3. The oxidizing power resulting from the union is somewhat more, or somewhat less, than that of either of the gametes.

in the egg as an index, measure, or determiner of the tyrosin oxidation powers of the adult. Secrets of protoplasmic differentiation, zymogenesis, stereochemistry, and catalytic action, all block for the present the tracing or predication of any such relations.

Undoubtedly enzymes have a very considerable importance in development. By focusing attention too closely upon them it is possible, however, to underestimate the importance of other, and even of related phenomena in which the *possibility* of "inherited specificity" is entirely eliminated. I refer particularly to the "autocatalysis" of such substances as oils studied by Guenthe ('07), Mathews, Walker and the writer ('08*b*), and others.

4. The result of the union is a blend; *i. e.*, an oxidizing power intermediate to that of the two gametes.

Numbers 1 and 2 represent colors at points of fairly fixed color equilibrium, as proved by the fact that individuals, varieties and species tend to stop color formation at those points; and most of the offspring of such hybrids may reasonably be expected to breed true with reference to this character, because of such stable equilibrium. Categories 3 and 4 often represent, on the other hand, colors at points of unfixed equilibrium; stages in the oxidation of tyrosin are not easily held at these points; that such points of unstable equilibrium arise in the chemical building of melanin, as elsewhere, is practically certain; this unstable condition is followed by an immediate tendency — in the second (next) generation usually — to shift to one or both of the stable points represented by the male and female condition, or to a new point.[1]

The above considerations seem to be of far-reaching applicability. They are, I think, rigorously consistent with what we know of the oxidation process, and with the various facts of melanogenesis; whereas Mendelian interpretation is consistent with neither. How much of the totality of color-inheritance is thus brought under one point of view will be at once appreciated by naturalists; while in striking contrast is the very small fraction of such inheritance that can be brought into the Mendelian system, even with all its elaborations.

In following out the implications of our conception of the state in which these color characters exist in the germ, it may be said that hybrid offspring possessing a color of easy, fixed equilibrium, mated with similar forms may usually be expected to breed true (that is, to continue this oxidizing power into their germ cells) with respect to this character. If mated, however, with another variety possessing some very different character, let us say size, which is also in very stable, fixed equilibrium, it seems quite

[1] These four types give nothing of *qualitativeness* nor of *discontinuity;* we have to deal absolutely in these initial stages with *quantity*, degree or pitch of oxidation power, and the gaps which we find in the end-result of the development of the color characters are but the cumulative, final expressions of different degrees of oxidation power, and of the fact that certain stages of oxidation are more stable — in firmer equilibrium — than others.

conceivable — almost a necessity — that each variety should at least sometimes be able to force its stable character (if these do not both rest directly upon the same process, *e. g.*, tyrosin oxidation) practically unchanged into a new combination and produce a new form — an animal with the *color* of one parent and the *size* of the other.

This clearly brings us face to face with something resembling unit characters and particulate inheritance. I can see no reason, from studies on the nature of color formation, and from the necessary deductions as to the way in which color must be transmitted, to doubt the possibility or the probability of the formation of races with new characters, or rather a race with a new combination of old characters; and with some such newly combined characters in very stable equilibrium, *i. e.*, breeding true. In fact, it is the recognition of the state in which our color character exists in the germ, that is, as a given pitch of power for oxidizing tyrosin compounds, and this not latent but active in the germ as later, that enables us to view the mechanism by which such a result is brought about.

The demonstration of the existence of such combinations of characters is, I believe, the supreme contribution of Mendelism to our knowledge of heredity. Phenomena of dominance, of segregation (?) and proportion, are but minor and special manifestations of a process much more important, general and inclusive; and which general process is, in color inheritance at any rate, the propagation and occasional quantitative modification (four types above) of oxidizing powers, with their more or less constancy of expression through settling into points of easy or fixed oxidizing equilibrium.

Our view does not, however, allow the acceptance of the unit character hypothesis without very considerable and rather radical modification. The prevailing idea among Mendelian workers has been essentially that each character, each recognizable differentiation, each member of a group of factors that forms a character, is quite separate and capable of being shuffled in the germs, and of independent appearance in the zygote. Now, as already noted, experience with the melanins drives home the point that a long series of very distinct characters have not each even *one*

representative, but *all* together have one basis in the germ — a power to oxidize tyrosin compounds, and this capable of close and continuous gradations. Many other characters, moreover, may also be closely connected genetically with the tyrosin oxidizing power of the organism. This being true, it is easily seen that what has been quite generally taken to be a "unit character" among colors cannot be in fact a "unit" of *modification.* All tyrosin oxidations, and some others as well, may be, and probably are, modified simultaneously and in corresponding manner.

The conception of "units of modification," then, which we adopt as an apparent logical necessity cannot regard as "units" the things that have been called unit characters in the past; quite certainly some unsound criteria, a false interpretation, a misleading nomenclature, and some observed facts are all back of the old conception as it has been applied to color behavior. We need not be surprised if it be found to embrace very little truth. To determine what a real "unit of modification" is, would seem to be no easy matter. Present Mendelian methods will doubtless contribute something, though not nearly all, to the complete story. For if — and there is little doubt — other definitive characters of the soma are likewise present in the germ only as varying "strengths" of rather general powers or processes, these same powers will exercise influence directly and indirectly, and to greater or less extent, in many and diverging directions during development; so that a "unit of modification" in inheritance would, in the broadest sense, include all such effects (there would probably be found all gradations of such effects). Obviously, therefore, *individual characters are by no means units of inheritance or modification.* And in any exhaustive search for such influence or modification it is quite possible that vision will sometimes be forced to cover the whole field from antibodies and immunity to size and color; from the grossest structural modification to the most delicate functional idiosyncrasy.

The question will be asked — how may those who reject the Mendelian interpretation based on representative particles, account for the segregation and proportions observed in Mendelian behavior? To undertake a discussion of this point is obviously

to leave the province of fact, with which the body of this paper deals to enter the realm of assumption and hypothesis. The fact, that in these pages we have been able to get what we are inclined to consider the clearest view that we have at present as to the state in which a character or set of characters exists in the germ, perhaps furnishes a reason why some statement may here be made in regard to the *possible mechanism of segregation.*

It has already been stated that to us the phenomena of dominance and segregation are only minor, surface, and incidental phenomena of heredity;[1] the really important Mendelian contribution being that certain different characters (such as have, according to my belief, different rather general processes as a basis) of different races may be combined to form new fixed races. The establishment of this last-named fact has been most commonly considered by Mendelians as, on the one hand, a consequence of the laws of dominance and segregation, and, on the other hand, as a strong argument for a "representative particle" basis for these two sets of phenomena. When, however, we learn that a certain character has no other existence in the germ than a rather general protoplasmic power, the "mnémon" conception fails completely and with it the supposed mechanism of its segregation — *i. e.*, the shuffling of mnémons in the reduction divisions of the chromosomes.

How then on my view of the basis of color inheritance may the segregation and proportions which result in Mendelian behavior be accounted for? I may say at once that I do not know; but in this respect I consider myself hardly worse off than the wisest Mendelian. My *supposition*, however, would put less faith than is theirs in the behavior of chromosomes during the

[1] It would seem that instead of viewing the real and entire sea of heredity, learning of its intimate nature, searching its boundaries, sounding its depths, too often Mendelians have — to indulge a figure — focused their visual instruments upon an optical section lying perhaps a few meters *above* sea-level. Here, occasionally, beautiful and regular phenomena come into sight; but for the most part the field is blank. At times great ocean swells pass in fine order and precision, permitting the observer to predict quite well some of the attributes of the next undulation and the time of its appearance; again, bits of spray or foam or mist may sometimes come into view, and the seeming disconnectedness of it all permits the be-focused onlooker to name and classify — and wonder. All — while the great ocean of heredity with its perfect continuities, its essential oneness, its inclusiveness, lies in unseen constancy and majesty beneath.

maturation divisions. It may for the present be assumed, and this is, I think, all that is really demanded in accounting for the differences in end-result in color development and inheritance — that the germ cells vary in "strength," *i. e.*, in such general powers as assimilation, growth, oxidation, etc. (and this proposition is not all assumption); this general difference of "strength" of germ cells may (or may not) arise during the maturation divisions; one or more of the four cells receiving in some species, though not in all, a type of protoplasm in better or worse condition than the others — influenced, for example, by more or less yolk; yolk more or less affected by previous nuclear and cytoplasmic contact; by variable distribution of the protoplasm of the astrosphere; by variable admixtures of nuclear (not necessarily chromatin) and cytoplasmic matter; by receiving more or fewer entire chromosomes, etc. (Chromatin is well known to be a very reactive substance and we may very well believe that wide variations of it in amount influence the vigor or intensity of vital processes occurring in a cell and in its derivatives; but in this respect chromatin is not unique, the other variables just mentioned doubtless do the same.) Any or all of these things will influence the development of a character only by serving to strengthen or weaken some *process* which underlies its formation.[1]

Such general differences of germ cells as arise from the several possible causes mentioned above would conceivably tend to affect the strength of such a general process as oxidation — such as produces scales of color. From the nature of these differences it will be seen that the growth and maturation stages may occasionally among organisms — constantly perhaps in some forms — furnish the conditions for the production of four sister sperm cells of unequal strength, one or two may be especially favor-

[1] It is perhaps well in our estimation of the basis for segregation of color characters, which rest directly on an oxidation process, to call attention to the special significance of the centrosome and cytaster. Considerable evidence is adduced by Mathews ('07) to show that the centrosome is the reduction center of the cell. If this should prove true, very obviously our estimation of the oxidation powers of the cell would be better treated in relation to this body than to any other in the cell. It cannot be too much emphasized, however, that upon this view the centrosome and the sphere is but a *region* where intense reduction occurs; the intensity grading off from centriole to (presumably) the periphery of the cell; and this region should be thought of as an expression of general powers of the whole cell.

ably equipped by the distribution of such materials, while one or two are left poorer than the others.[1] A similar conception may be applied to the ova. Then upon the union of male and female cell, two oxidizing powers of equal or unequal rank, of higher or of lower degree, meet. A stable (pure breed) high equilibrium results in, say one of four; a stable (pure breed) lower equilibrium results in another; while in the other two perfect equilibrium is not at once attained.

Here is then a *possible* picture of the basis of Mendelian segregation and proportion, but without recourse to hypothetical "particles" or to immutable and immortal factors. An apparently very specific end-result of an oxidation would be traceable in the germ only in the strength or pitch of a general vital process, and not at all in mnémons or representative particles packed with unthinkable precision, order and potentiality into (presumably) the chromosomes. But the above is a possible picture only; and it is not here my purpose to furnish nor to defend at length a possible or probable theory of the mechanism of heredity. The material in hand lends itself first of all to the demonstration of the *impossibility* of many Mendelian views.

The nature of present Mendelian interpretation and description inextricably commits to the "doctrine of particles" in the germ and elsewhere. It demands a "morphological basis" in the germ for the minutest phase (factor) of a definitive character. It is essentially a morphological conception with but a trace of functional feature. Although heredity is quite surely a functional process of major complexity, it may be recalled that the primary and fundamental Mendelian conception of this process utilizes not a single finding of the science of biochemistry; that the only physiological fact utilized is the one of certain occasionally observed segregation behavior which is exhibited in the end-results of varietal[2] or specific character formation; such segregation, by

[1] We know that in the corresponding divisions of *ovogenesis* that extremely disproportionate distribution of yolk and cytoplasm occurs quite constantly. We may believe that the laws which there give rise to the *extreme* differences between polar body and oöcyte are not *everywhere else*, and *completely*, unoperative; they may be operative — though in a much less pronounced degree — in providing different *mature* ova, and different sperms with varying amounts of the materials mentioned above.

[2] De Vries ('01, '05) has asserted that only varietal, not specific, differences exhibit Mendelian heredity; this statement is not accepted by Bateson ('06), and is controverted by still other workers.

inference, arising from the temporary mixture (heterozygote), or failure to mix (homozygote) in the gamete, of something, no one knew what — but which has been generally conceived of as some sort of "particles." (In *later* additions to, and *special* applications of the Mendelian conception, certain other biochemical and physiological facts have, of course, been considered.)

This is precisely why present Mendelian interpretation and description of heredity is a bar to the progress of studies in inheritance and development; with an eye seeing only *particles*, and a speech only symbolizing them, there is no such thing as the study of a *process* possible.

The conception that organic color has at its basis not rigid, immortal particles, but yielding, equilibrium-seeking powers, or strengths of processes, makes the infinite variety of colors in organisms intelligible. If, on the other hand, particles, and a mechanism for their continual segregation and propagation pure were in reality at the basis of color inheritance, we should rather expect uniformity, not the actual diversity, to be the dominant feature of organic coloration. Indeed, a modification of the strength of many organic processes (and so of color formation) would be a necessary accompaniment — a result, even if not a cause — of that "transformation of organs" which has been the very labor of phylogenetic development. The atrophy, superior development and transformation of organs are certainly efficient factors in such modification, for it is a physiological fact of common experience that many, or most, of the vital processes are not equally strong or pronounced in all of the organs of the same organism; and that many metabolic processes of the body are dependent upon special organs for their highest expression, for the completest manifestation of their power.

Let me not be understood to say that our knowledge of the development of melanin color characters is complete. There is much yet to be learned. But the significant thing about it is that we now know *so much* of the mechanism of the building of these characters in comparison with what we know of a similar nature in non-color characters. It has been possible, I think, to show by means of what we know of the genesis of these color characters that the Mendelian description — of color inheritance

at least — has strayed very wide of the facts; it has put factors in the germ cells that it is now quite certainly our privilege to remove; it has declared discontinuity where there is now proved continuity; it has postulated preformation where there is now evident epigenesis.

Is it too much to expect that the further application of such tests as the one here presented in outline for the melanin colors will in the end remove *many* of the Mendelian "factors" from the germ cells? That many of their "characters" will come to rest on a more proximate basis; will be known to have their "determination" and origin in very general germinal powers, and in somatic conditions obtaining previous to, or at the time of, their development? Will not other Mendelian discontinuities then begin to disclose gradations, and other qualitative differences then appear more and more as quantitative sequences?

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